| | Type | L # | Hits | Search Text | DBs | Time Stamp | Comments | Error Definition | Errors |
|---|---|---|---|---|---|---|---|---|---|
| 1 | BRS | L1 | 6 | (CXCR2 or CXCR1) near3 receptor$ | USPAT | 2001/06/15 15:43 | | | 0 |
| 2 | BRS | L2 | 11 | (CXCR2 or CXCR1) | USPAT | 2001/06/15 15:43 | | | 0 |

```
=> s (CXCR2 or IL(2a)8B)
L1          300 (CXCR2 OR IL(2A) 8B)

=> s l1 and (chemotax? or neutrophil?) and (inhibit? or block? or antagoniz?)
L2           83 L1 AND (CHEMOTAX? OR NEUTROPHIL?) AND (INHIBIT? OR BLOCK? OR
                ANTAGONIZ?)

=> s CXCR2
L3          278 CXCR2

=> s l3 and (chemotax? or neutrophil?) and (inhibit? or block? or antagoniz?)
L4           76 L3 AND (CHEMOTAX? OR NEUTROPHIL?) AND (INHIBIT? OR BLOCK? OR
                ANTAGONIZ?)

=> s (CXCR2 or IL(2a)8RB)
L5          339 (CXCR2 OR IL(2A) 8RB)

=> s l5 and (chemotax? or neutrophil?) and (inhibit? or block? or antagoniz?)
L6          112 L5 AND (CHEMOTAX? OR NEUTROPHIL?) AND (INHIBIT? OR BLOCK? OR
                ANTAGONIZ?)

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7          112 DUP REM L6 (0 DUPLICATES REMOVED)

=> s l7 and (inflammat? or arthriti? or reperfus? or atherosclero? or asthma or
chemokine#)
L8          107 L7 AND (INFLAMMAT? OR ARTHRITI? OR REPERFUS? OR ATHEROSCLERO?
                OR ASTHMA OR CHEMOKINE#)

=> s l8 and py <1998
L9           20 L8 AND PY <1998

=> d l9 abs ibib kwic 1-20

L9   ANSWER 1 OF 20  CAPLUS  COPYRIGHT 2002 ACS
AB   The CXC-chemokines interleukin-8 (IL-8), neutrophil
     -activating peptide-2 (NAP-2), and melanoma growth-stimulatory activity
     (MGSA) are chemoattractants with high selectivity for neutrophils
     . Although IL-8 has been shown to act as an extremely potent mediator,
     reports on NAP-2 and MGSA are still contradictory. Here the authors show
     for the first time that NAP-2 and MGSA induce two distinct optima of
     neutrophil chemotaxis. A first optimum is elicited
     within a concn. range as low as is characteristic for IL-8. However, a
     second optimum appears at more than 200-fold higher stimulus concns., at
     which IL-8 is inactive. Investigating the involvement of the two
     chemokine receptors CXCR-1 and CXCR-2 in NAP-2-mediated
     chemotaxis, the authors observe that the cells become desensitized
     to the first optimum of the chemokine after selective
     downregulation of CXCR-2, while both optima disappear upon simultaneous
     downregulation of both receptors. Blocking monoclonal
     antibodies (MoAbs) specific for CXCR-2 or CXCR-1 either suppress the first
     optimum of NAP-2-induced chemotaxis or drastically reduce the
     second one, resp. These results provide evidence that both receptors are
     involved in NAP-2-induced neutrophil chemotaxis, with
     CXCR-2 rendering the cells responsive to low dosages of the
     chemokine, and with CXCR-1 extending their responsiveness to NAP-2
```

dosages higher by several orders of magnitude.

ACCESSION NUMBER: 1997:771619 CAPLUS
DOCUMENT NUMBER: 128:60562
TITLE: The CXC-**chemokine neutrophil** -activating peptide-2 induces two distinct optima of **neutrophil chemotaxis** by differential interaction with interleukin-8 receptors CXCR-1 and CXCR-2
AUTHOR(S): Ludwig, Andreas; Petersen, Frank; Zahn, Stefan; Gotze, Otto; Schroder, Jens-Michael; Flad, Hans-Dieter; Brandt, Ernst
CORPORATE SOURCE: Department of Immunology and Cell Biology, Forschungszentrum Borstel, Borstel, D-23845, Germany
SOURCE: Blood (**1997**), 90(11), 4588-4597
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

TI The CXC-**chemokine neutrophil**-activating peptide-2 induces two distinct optima of **neutrophil chemotaxis** by differential interaction with interleukin-8 receptors CXCR-1 and CXCR-2

SO Blood (**1997**), 90(11), 4588-4597
CODEN: BLOOAW; ISSN: 0006-4971

AB The CXC-**chemokines** interleukin-8 (IL-8), **neutrophil** -activating peptide-2 (NAP-2), and melanoma growth-stimulatory activity (MGSA) are chemoattractants with high selectivity for **neutrophils** . Although IL-8 has been shown to act as an extremely potent mediator, reports on NAP-2 and MGSA are still contradictory. Here the authors show for the first time that NAP-2 and MGSA induce two distinct optima of **neutrophil chemotaxis**. A first optimum is elicited within a concn. range as low as is characteristic for IL-8. However, a second optimum appears at more than 200-fold higher stimulus concns., at which IL-8 is inactive. Investigating the involvement of the two **chemokine** receptors CXCR-1 and CXCR-2 in NAP-2-mediated **chemotaxis**, the authors observe that the cells become desensitized to the first optimum of the **chemokine** after selective downregulation of CXCR-2, while both optima disappear upon simultaneous downregulation of both receptors. **Blocking** monoclonal antibodies (MoAbs) specific for CXCR-2 or CXCR-1 either suppress the first optimum of NAP-2-induced **chemotaxis** or drastically reduce the second one, resp. These results provide evidence that both receptors are involved in NAP-2-induced **neutrophil chemotaxis**, with CXCR-2 rendering the cells responsive to low dosages of the **chemokine**, and with CXCR-1 extending their responsiveness to NAP-2 dosages higher by several orders of magnitude.

ST **neutrophil** activating peptide 2 **neutrophil** **chemotaxis**; melanoma growth stimulatory activity **neutrophil chemotaxis**; **neutrophil** **chemotaxis** NAP 2 MGSA receptor; CXCR1 **neutrophil** **chemotaxis** NAP 2 MGSA; **CXCR2 neutrophil** **chemotaxis** NAP 2 MGSA

IT **Neutrophil chemotaxis**
(**neutrophil**-activating peptide-2 and melanoma growth-stimulatory activity induction of human **neutrophil** **chemotaxis** by differential interaction with CXCR-1 and CXCR-2 receptors)

IT MGSA **chemokine**
**Neutrophil**-activating peptide-2

RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(**neutrophil**-activating peptide-2 and melanoma growth-stimulatory activity induction of human **neutrophil** **chemotaxis** by differential interaction with CXCR-1 and CXCR-2 receptors)

IT Interleukin 8 receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.alpha.; **neutrophil**-activating peptide-2 and melanoma growth-stimulatory activity induction of human **neutrophil** **chemotaxis** by differential interaction with CXCR-1 and CXCR-2 receptors)

IT Interleukin 8 receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.beta.; **neutrophil**-activating peptide-2 and melanoma growth-stimulatory activity induction of human **neutrophil** **chemotaxis** by differential interaction with CXCR-1 and CXCR-2 receptors)

L9 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB The authors studied the pathways that lead to arrest and firm adhesion of rolling PMN on activated, surface-adherent platelets. Stable arrest and adhesion strengthening of PMN on thrombin-stimulated, surface-adherent platelets in flow required distinct Ca2+- and Mg2+-dependent regions of Mac-1 (.alpha.M.beta.2), and involved interactions of Mac-1 with fibrinogen, which was bound to platelets via .alpha.IIb.beta.3. Mac-1 also bound to other unidentified ligands on platelets, which were not intracellular adhesion mol.-2 (ICAM-2), heparin, or heparan-sulfate proteoglycans. This was shown by **inhibition** with mAbs or peptides, by treatment of platelets with heparitinase, and by using platelets with defective .alpha.IIb.beta.3 from a patient with Glanzmann thrombasthenia. Tethering of PMN on platelet ICAM-2 via LFA-1 (.alpha.L.beta.2) was obsd., which may facilitate the transition between rolling on selectins and Mac-1-dependent arrest. Arrest and adhesion strengthening was pertussis toxin sensitive and in flow was mainly induced by platelet-activating factor but not through activation of the **chemokine** receptor **CXCR2**. In stasis, spreading occurred and the **CXCR2** contributed to firm adhesion.

ACCESSION NUMBER: 1997:676789 CAPLUS
DOCUMENT NUMBER: 127:345299
TITLE: **Neutrophil** accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to .alpha.IIb.beta.3 and stimulated by platelet-activating factor
AUTHOR(S): Weber, Christian; Springer, Timothy A.
CORPORATE SOURCE: The Center for Blood Research and Department of Pathology, Harvard Medical School, Boston, MA, 02115, USA
SOURCE: J. Clin. Invest. (**1997**), 100(8), 2085-2093
CODEN: JCINAO; ISSN: 0021-9738
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

TI **Neutrophil** accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to .alpha.IIb.beta.3 and stimulated by platelet-activating factor

SO J. Clin. Invest. (**1997**), 100(8), 2085-2093

CODEN: JCINAO; ISSN: 0021-9738

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ST **neutrophil** platelet Mac1 fibrinogen alphaIIbbeta3 PAF; integrin platelet activating factor **neutrophil** accumulation

IT Cytokine receptors
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(**chemokine**, **CXCR2**; **neutrophil** accumulation in flow involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

IT Cell adhesion
**Neutrophil**
Platelet (blood)
(**neutrophil** accumulation in flow involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

IT Integrin .alpha.IIb.beta.3
Mac-1 antigen
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(**neutrophil** accumulation in flow involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

IT ICAM-2 (cell adhesion molecule)
LFA-1 (antigen)
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(**neutrophil** accumulation in flow involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

IT Fibrinogens
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(**neutrophil** accumulation in flow involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

IT **Chemokines**
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(receptors, **CXCR2**; **neutrophil** accumulation in flow

involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

IT **Neutrophil** infiltration
(recruitment; **neutrophil** accumulation in flow involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

IT 65154-06-5, Platelet-activating factor
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(**neutrophil** accumulation in flow involves interaction of Mac-1 with fibrinogen presented by .alpha.IIb.beta.3 on platelets, activation by platelet-activating factor, and tethering on ICAM-2)

L9 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB Interleukin-8 (IL-8), a CXC **chemokine**, acts on **neutrophils** through high-affinity binding to at least two distinct receptor classes, CXCR1 and **CXCR2**, both of which are G-coupled, 7-helix transmembrane receptors. Antibodies which bind IL-8 and **block** its binding to receptors have demonstrated that **blocking** the interaction of this ligand with its receptors can effectively **inhibit neutrophil** recruitment in conditions of **inflammation**. We have sought to understand the details of IL-8: receptor interaction in order to design small-mol. **inhibitors** of this process. From mutagenesis studies of IL-8, it has become clear that the natural interface consists of multiple ligand-receptor contacts. We have further explored the role of IL-8 self-assocn. through a series of variants which alter the dimerization affinity of the ligand without significantly perturbing the interaction with receptor. These in turn suggest new approaches to the design of IL-8 antagonists.

ACCESSION NUMBER: 1997:489895 CAPLUS
TITLE: Stoichiometry and binding interactions in the interleukin-8 **inflammation** pathway.
AUTHOR(S): Lowman, H. B.
CORPORATE SOURCE: Department Protein Engineering, Genentech, Inc., South San Francisco, CA, 94080, USA
SOURCE: Book of Abstracts, 214th ACS National Meeting, Las Vegas, NV, September 7-11 (**1997**), MEDI-138. American Chemical Society: Washington, D. C. CODEN: 64RNAO
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English

TI Stoichiometry and binding interactions in the interleukin-8 **inflammation** pathway.

SO Book of Abstracts, 214th ACS National Meeting, Las Vegas, NV, September 7-11 (**1997**), MEDI-138 Publisher: American Chemical Society, Washington, D. C.
CODEN: 64RNAO

AB Interleukin-8 (IL-8), a CXC **chemokine**, acts on **neutrophils** through high-affinity binding to at least two distinct receptor classes, CXCR1 and **CXCR2**, both of which are G-coupled, 7-helix transmembrane receptors. Antibodies which bind IL-8 and **block** its binding to receptors have demonstrated that **blocking** the interaction of this ligand with its receptors can effectively **inhibit neutrophil** recruitment in conditions of **inflammation**. We have sought to understand the

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L9 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB Human **neutrophils** express two interleukin (IL)-8 receptors, CXC **chemokine** receptor (CXCR) 1 and **CXCR2**. IL-8, with changes to the NH2-terminal ELR motif, can **block** IL-8-induced **neutrophil** functions (Moser, B., Dewald, B., Barella, L., Schumacher, C., Baggiolini, M., and Clark-Lewis, I. (1993) J. Biol. Chem. 268, 7125-7128). We have now examd. the effect of NH2-terminally modified analogs of IL-8, GRO.alpha., and PF4 on CXCR1 and CRCR2 independently. Using stable Jurkat transfectants expressing either CXCR1 or **CXCR2**, it was shown that analogs derived from IL-8 bound both IL-8 receptors with similar affinity and could **block** IL-8-induced Ca2+ mobilization. By contrast, analogs of GRO.alpha. and PF4, (R)GRO.alpha. and (R)PF4, bound only **CXCR2** with high affinity and **blocked** Ca2+ mobilization induced only via **CXCR2**. The differential effect on CXCR1 and **CXCR2** was also demonstrated in studies with isolated **neutrophils**. Thus (R)GRO.alpha. and (R)PF4 **inhibited** only the GRO.alpha. but not the IL-8-stimulated elastase release, and these two analogs had no effect on IL-8-elicited superoxide generation, a response that is mediated by CXCR1 but not by **CXCR2**. These results show that **CXCR2** selective receptor antagonists can be generated based upon the secondary binding determinants of GRO.alpha. and PF4. They also highlight the primary importance of CRCR1 in **chemokine**-mediated release of granule enzymes and superoxide generation. The selective antagonists described may be used in future studies on IL-8 receptor signaling to define distinct steps leading to various functional responses induced in **neutrophils** via CXCR1 and **CXCR2**.

ACCESSION NUMBER: 1997:421997 CAPLUS
DOCUMENT NUMBER: 127:160356
TITLE: **Chemokine** antagonists that discriminate between interleukin-8 receptors. Selective **blockers** of **CXCR2**
AUTHOR(S): Jones, Simon A.; Dewald, Beatrice; Clark-Lewis, Ian; Baggiolini, Marco
CORPORATE SOURCE: Theodor-Kocher Institute, University Bern, Bern, CH-3000, Switz.
SOURCE: J. Biol. Chem. (**1997**), 272(26), 16166-16169 CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

TI **Chemokine** antagonists that discriminate between interleukin-8 receptors. Selective **blockers** of **CXCR2**

SO J. Biol. Chem. (**1997**), 272(26), 16166-16169 CODEN: JBCHA3; ISSN: 0021-9258

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**neutrophil** functions (Moser, B., Dewald, B., Barella, L., Schumacher, C., Baggiolini, M., and Clark-Lewis, I. (1993) J. Biol. Chem. 268, 7125-7128). We have now examd. the effect of NH2-terminally modified analogs of IL-8, GRO.alpha., and PF4 on CXCR1 and CRCR2 independently. Using stable Jurkat transfectants expressing either CXCR1 or **CXCR2**, it was shown that analogs derived from IL-8 bound both IL-8 receptors with similar affinity and could **block** IL-8-induced Ca2+ mobilization. By contrast, analogs of GRO.alpha. and PF4, (R)GRO.alpha. and (R)PF4, bound only **CXCR2** with high affinity and **blocked** Ca2+ mobilization induced only via **CXCR2**. The differential effect on CXCR1 and **CXCR2** was also demonstrated in studies with isolated **neutrophils**. Thus (R)GRO.alpha. and (R)PF4 **inhibited** only the GRO.alpha. but not the IL-8-stimulated elastase release, and these two analogs had no effect on IL-8-elicited superoxide generation, a response that is mediated by CXCR1 but not by **CXCR2**. These results show that **CXCR2** selective receptor antagonists can be generated based upon the secondary binding determinants of GRO.alpha. and PF4. They also highlight the primary importance of CRCR1 in **chemokine**-mediated release of granule enzymes and superoxide generation. The selective antagonists described may be used in future studies on IL-8 receptor signaling to define distinct steps leading to various functional responses induced in **neutrophils** via CXCR1 and **CXCR2**.

ST human **neutrophil** IL8 receptor analog GROalpha; CXCR1 receptor binding analog IL8 ***neutrophil*** ; **CXCR2** receptor binding analog PF4 **neutrophil**

IT Interleukin 8
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
((AAA)IL-8 and (R)IL-8 and (R)IL-8,NMeLeu; anal. of N-terminally modified analogs of IL-8, GRO.alpha. and PF4 for **inhibition** of **neutrophil** function and selective binding to CXCR1 and **CXCR2** bearing cells)

IT MGSA **chemokine**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
((R)GRO.alpha.; anal. of N-terminally modified analogs of IL-8, GRO.alpha. and PF4 for **inhibition** of **neutrophil** function and selective binding to CXCR1 and **CXCR2** bearing cells)

IT **Neutrophil**
(anal. of N-terminally modified analogs of IL-8, GRO.alpha. and PF4 for **inhibition** of **neutrophil** function and selective binding to CXCR1 and **CXCR2** bearing cells)

IT Interleukin 8 receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.alpha., renamed CXCR1 (CXC **chemokine** receptor 1); anal. of N-terminally modified analogs of IL-8, GRO.alpha. and PF4 for **inhibition** of **neutrophil** function and selective binding to CXCR1 and **CXCR2** bearing cells)

IT Interleukin 8 receptors
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(.beta., renamed **CXCR2** (CXC **chemokine** receptor 2); anal. of N-terminally modified analogs of IL-8, GRO.alpha. and PF4 for **inhibition** of **neutrophil** function and selective binding to CXCR1 and **CXCR2** bearing cells)

IT 37270-94-3, Platelet factor 4

RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
((ELR)PF4 and (R)PF4; anal. of N-terminally modified analogs of IL-8, GRO.alpha. and PF4 for **inhibition** of **neutrophil** function and selective binding to CXCR1 and **CXCR2** bearing cells)

IT 9004-06-2, Elastase
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(effect of IL-8 antagonists on elastase release induced in human **neutrophils** by IL-8 and GRO.alpha.)

IT 11062-77-4, Superoxide
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(effect of IL-8 antagonists on superoxide prodn. by human **neutrophils**)

L9 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB Two receptors for interleukin 8 (IL-8), IL-8rA and **IL-8rB**, have been cloned. Previous studies of **neutrophils** indicated that the two C-X-C **chemokines**, IL-8 and NAP-2, bind to **IL-8rB** with high affinity but that only IL-8 binds to IL-8rA with high affinity. In this study, human kidney embryonal 293 cells were transfected to express solely IL-8rA or **IL-8rB** (the cells are designated IL-8rA/293 and **IL-8rB**/293, resp.). The authors show that NAP-2 bound both IL-8rA and **IL-8rB** specifically. While NAP-2 and IL-8 bound **IL-8rB** with comparable high affinity (2.9 and 2.8 nM, resp.), NAP-2 showed a lower binding affinity to IL-8rA (9 nM) compared with IL-8 (1.3 nM). A lower no. of binding sites was detected for NAP-2 than for IL-8 on IL-8rA/293 cells as well on **IL-8rB** /293 cells. On both cell types (IL-8rA/293 and **IL-8rb** /293), NAP-2 and IL-8 could completely **inhibit** [125I]NAP-2 binding, while unlabeled NAP-2 could only partially compete for [125I]IL-8 binding. Functional assays revealed that although NAP-2 is chemotactic for both IL-8rA/293 and **IL-8rB**/293 cells, it is less potent than IL-8. While NAP-2 induced **chemotaxis** of **IL-8rB**/293 cells at the same optimal concns. as IL-8 (10-100 ng/mL), the induction of optimal migratory response of IL-8rA/293 cells required much higher concns. of NAP-2 than IL-8 (1000-3000 ng/mL and 10-100 ng/mL, resp.). The dose-response curve of the **IL-8rB**/293 cells to IL-8 was bell shaped, while the response to NAP-2 was sustained at a plateau level even at concns. as high as 3000 ng/mL. It is likely that tertiary structural differences between NAP-2 and IL-8 account for their divergent abilities to bind and chemoattract 293 cells transfected with either IL-8 receptor type A or type B.

ACCESSION NUMBER: 1997:173412 CAPLUS
DOCUMENT NUMBER: 126:250007
TITLE: IL-8 and NAP-2 differ in their capacities to bind and chemoattract 293 cells transfected with either IL-8 receptor type A or type B
AUTHOR(S): Ben-Baruch, Adit; Bengali, Kathleen; Tani, Kenji; Xu, Luoling; Oppenheim, Joost J.; Wang, Ji Ming
CORPORATE SOURCE: Natl. Cancer Institute-Frederick, Cancer Research and Development Center, Frederick, MD, USA
SOURCE: Cytokine (**1997**), 9(1), 37-45
CODEN: CYTIE9; ISSN: 1043-4666
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

SO Cytokine (**1997**), 9(1), 37-45
CODEN: CYTIE9; ISSN: 1043-4666

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ST interleukin 8 **neutrophil** activating peptide 2

IT **Chemotaxis**
(chemotactic effects of interleukin-8 and **neutrophil** activating peptide 2 in relation to interleukin-8 receptor types A and B)

IT Interleukin 8
**Neutrophil**-activating peptide-2
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(interleukin-8 and **neutrophil** activating peptide 2 differ in their capacities to bind interleukin-8 receptor types A and B)

IT Interleukin 8 receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.alpha.; interleukin-8 and **neutrophil** activating peptide 2 differ in their capacities to bind interleukin-8 receptor types A and B)

IT Interleukin 8 receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(.beta.; interleukin-8 and **neutrophil** activating peptide 2 differ in their capacities to bind interleukin-8 receptor types A and B)

L9 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB Human granulocyte chemotactic protein 2 (GCP-2) has originally been isolated from cytokine-stimulated osteosarcoma cells as a **chemokine** coproduced in minute amts. together with interleukin 8. Human GCP-2 (75 residues) was synthesized on a 0.25-mmol scale using Fmoc chem. After disulfide bridge formation and purifn., monomeric GCP-2 was

recovered as a 6-kDa protein; the pure synthetic protein showed a mol. mass of 8076 Da as detd. by matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS). The exact amino acid sequence of synthetic GCP-2 was confirmed by Edman degrdn. Synthetic GCP-2 was an equally active (minimal effective concn. of 1-3 nM) chemoattractant for **neutrophilic** granulocytes as was natural 75-residue GCP-2. At concns. up to 30 nM, synthetic GCP-2 did not stimulate eosinophil, monocyte, or lymphocyte **chemotaxis**. GCP-2 induced a dose-dependent increase in [Ca2+]i in **neutrophils**, 1 nM being the minimal effective concn. The GCP-2-induced [Ca2+]i increase was completely prevented by pertussis toxin. Prestimulation of **neutrophils** with equimolar concns. of purified natural IL-8, GRO.alpha., GRO.gamma., and ENA-78 abolished the [Ca2+]i increase in response to 1 nM GCP-2. Alternatively, the [Ca2+]i rise induced by these CXC **chemokines** was **inhibited** by pretreatment of **neutrophils** with GCP-2. GCP-2 stimulated [Ca2+]i increases in CXCR1- and **CXCR2**-transfected cells, demonstrating that GCP-2 binds to both IL-8 receptors. Intradermal injection of synthetic GCP-2 resulted in a dose-dependent **neutrophil** accumulation and plasma extravasation in rabbit skin. To provoke this skin reaction, GCP-2 (10 pmol/site) was nearly as effective as IL-8, indicating that it is an important complementary mediator of the **inflammatory** response.

ACCESSION NUMBER: 1997:127209 CAPLUS
DOCUMENT NUMBER: 126:116950
TITLE: Characterization of Synthetic Human Granulocyte Chemotactic Protein 2: Usage of **Chemokine** Receptors CXCR1 and **CXCR2** and in Vivo **Inflammatory** Properties
AUTHOR(S): Wuyts, Anja; Van Osselaer, Nancy; Haelens, Annemie; Samson, Isabelle; Herdewijn, Piet; Ben-Baruch, Adit; Oppenheim, Joost J.; Proost, Paul; Van Damme, Jo
CORPORATE SOURCE: Laboratories of Molecular Immunology and Medicinal Chemistry Rega Institute for Medical Research, University of Leuven, Louvain, B-3000, Belg.
SOURCE: Biochemistry (**1997**), 36(9), 2716-2723
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Characterization of Synthetic Human Granulocyte Chemotactic Protein 2: Usage of **Chemokine** Receptors CXCR1 and **CXCR2** and in Vivo **Inflammatory** Properties

SO Biochemistry (**1997**), 36(9), 2716-2723
CODEN: BICHAW; ISSN: 0006-2960

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the minimal effective concn. The GCP-2-induced [Ca2+]i increase was completely prevented by pertussis toxin. Prestimulation of **neutrophils** with equimolar concns. of purified natural IL-8, GRO.alpha., GRO.gamma., and ENA-78 abolished the [Ca2+]i increase in response to 1 nM GCP-2. Alternatively, the [Ca2+]i rise induced by these CXC **chemokines** was **inhibited** by pretreatment of **neutrophils** with GCP-2. GCP-2 stimulated [Ca2+]i increases in CXCR1- and **CXCR2**-transfected cells, demonstrating that GCP-2 binds to both IL-8 receptors. Intradermal injection of synthetic GCP-2 resulted in a dose-dependent **neutrophil** accumulation and plasma extravasation in rabbit skin. To provoke this skin reaction, GCP-2 (10 pmol/site) was nearly as effective as IL-8, indicating that it is an important complementary mediator of the **inflammatory** response.

ST granulocyte chemotactic protein 2 CXCR1 **CXCR2**; **inflammation** granulocyte chemotactic protein 2 **chemokine**

IT **Chemokines**
RL: ADV (Adverse effect, including toxicity); BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(granulocyte chemotactic protein 2 (GCP-2); granulocyte chemotactic protein 2 is **neutrophil** chemoattractant that binds **chemokine** receptors CXCR1 and **CXCR2** and is **inflammatory**)

IT **Chemotaxis**
**Inflammation**
**Neutrophil**
(granulocyte chemotactic protein 2 is **neutrophil** chemoattractant that binds **chemokine** receptors CXCR1 and **CXCR2** and is **inflammatory**)

IT Interleukin 8 receptors
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(.alpha.; granulocyte chemotactic protein 2 is **neutrophil** chemoattractant that binds **chemokine** receptors CXCR1 and **CXCR2** and is **inflammatory**)

IT Interleukin 8 receptors
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
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L9 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB **Chemokines** bind and signal through G-protein coupled seven transmembrane receptors. Various **chemokine** receptors are expressed on leukocytes, and these may impart selective homing of leukocyte subsets to sites of **inflammation**. Human eosinophils express the eotaxin receptor, CCR3, but respond to a variety of CC **chemokines** apart from eotaxin, including RANTES, monocyte chemotactic protein (MCP)-2, MCP-3, and MCP-4. Here we describe a mAb, 7B11, that is selective for CCR3 and has the properties of a true receptor antagonist. The 7B11 **blocked** binding of various radiolabeled **chemokines** to either CCR3 transfectants, or eosinophils. Pretreatment of eosinophils with this mAb **blocked** **chemotaxis** and calcium flux induced by all CCR3 ligands. In all individuals examd., including allergic and eosinophilic donors, >95% of the response of eosinophils to eotaxin, RANTES , MCP-2, MCP-3, and MCP-4 was shown to be mediated through CCR3. The IL-8 receptors, particularly

**CXCR2**, were induced on IL-5 primed eosinophils, however these eosinophils responded to CC **chemokines** in the same manner as unprimed eosinophils. These results demonstrate the importance of CCR3 for eosinophil responses, and the feasibility of completely **antagonizing** this receptor.

ACCESSION NUMBER: 1997:70214 CAPLUS
DOCUMENT NUMBER: 126:143145
TITLE: **Chemokine** receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody
AUTHOR(S): Heath, Heidi; Qin, Shixin; Rao, Pat; Wu, Lijun; LaRosa, Greg; Kassam, Nasim; Ponath, Paul D.; Mackay, Charles R.
CORPORATE SOURCE: LeukoSite, Inc., Cambridge, MA, 02142, USA
SOURCE: J. Clin. Invest. (**1997**), 99(2), 178-184
CODEN: JCINAO; ISSN: 0021-9738
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

TI **Chemokine** receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody

SO J. Clin. Invest. (**1997**), 99(2), 178-184
CODEN: JCINAO; ISSN: 0021-9738

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ST eosinophil CCR3 **chemokine** receptor

IT RANTES (**chemokine**)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(CCR3 **chemokine** receptor usage by human eosinophils and response to)

IT **Chemotaxis**
Eosinophil
(**chemokine** receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody)

IT Monoclonal antibodies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**chemokine** receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody)

IT Cytokine receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**chemokine**, CCR3; **chemokine** receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody)
IT Cytokines
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(monocyte chemotactic factor, -2; CCR3 **chemokine** receptor usage by human eosinophils and response to)
IT Cytokines
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(monocyte chemotactic factor, -3; CCR3 **chemokine** receptor usage by human eosinophils and response to)
IT Cytokines
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(monocyte chemotactic factor, -4; CCR3 **chemokine** receptor usage by human eosinophils and response to)
IT **Chemokines**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(receptors, CCR3; **chemokine** receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody)
IT 7440-70-2, Calcium, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(release; **chemokine** receptor usage by human eosinophils: the importance of CCR3 demonstrated using an antagonistic monoclonal antibody)

L9 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2002 ACS
AB To date, the activities of the .alpha. **chemokines** for human peripheral B cells from normal subjects (N-B cells) or from HIV-infected subjects (HIV-B cells) are not well established. No report on the IL-8R expression on N-B cells and HIV-B cells has been seen. The authors report here that the .alpha. **chemokines** IL-8 and growth-regulatory oncogene-.alpha. (GRO-.alpha.) induce a chemotactic migration of N-B cells and HIV-B cells via stimulating the **IL-8RB** on these cells. The **chemotaxis** of N-B cells can be **inhibited** by IFN-.gamma. and IL-2, and augmented by IL-4 and IL-13, whereas TNF-.alpha. and IL-10 have no influence. The **chemotaxis** of HIV-B cells can be **inhibited** by IFN-.gamma. and IL-2, and augmented by TNF.alpha., IL-4, and IL-10, whereas IL-13 has no influence. IL-8R are expressed more abundantly on freshly isolated HIV-B cells than N-B cells (51% and 15%, resp.). The IL-8R on N-B cells can be down-regulated by IFN-.gamma., IL-2, and TNF-.alpha. (selectively on IL-8RA), and up-regulated by IL-4 and IL-13, whereas IL-10 has no influence. The IL-8R on HIV-B cells can be down-regulated by IFN-.gamma. and IL-2, and up-regulated by TNF-.alpha., IL-4, and IL-10, whereas IL-13 has no influence. Importantly, N-B cell and HIV-B cell **chemotaxis** toward IL-8 and GRO-.alpha. can be **blocked** by anti-**IL-8RB** polyclonal Ab, but not by anti-IL-8RA polyclonal Ab. Thus, IL-8 and GRO-.alpha. are important **inflammatory** mediators that stimulate the directional migration and recruitment of B lymphocytes. The migratory behavior and the expression of IL-8R on HIV-B cells and some of the reactions to Th1- and Th2-like cytokines are modified during HIV infection.
ACCESSION NUMBER: 1997:14287 CAPLUS
DOCUMENT NUMBER: 126:46225
TITLE: **Chemotaxis** and IL-8 receptor expression in B

cells from normal and HIV-infected subjects

AUTHOR(S): Jinquan, Tan; Moller, Bjarne; Storgaard, Merete; Mukaida, Naofumi; Bonde, Jesper; Grunnet, Niels; Black, Finn Trunk; Larsen, Christian Gronhoj; Matsushima, Kouji; et al.
CORPORATE SOURCE: Dep. Dermatology, Aarhus Univ., Aarhus C, DK-8000, Den.
SOURCE: J. Immunol. (**1997**), 158(1), 475-484
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

TI **Chemotaxis** and IL-8 receptor expression in B cells from normal and HIV-infected subjects

SO J. Immunol. (**1997**), 158(1), 475-484
CODEN: JOIMA3; ISSN: 0022-1767

AB To date, the activities of the .alpha. **chemokines** for human peripheral B cells from normal subjects (N-B cells) or from HIV-infected subjects (HIV-B cells) are not well established. No report on the IL-8R expression on N-B cells and HIV-B cells has been seen. The authors report here that the .alpha. **chemokines** IL-8 and growth-regulatory oncogene-.alpha. (GRO-.alpha.) induce a chemotactic migration of N-B cells and HIV-B cells via stimulating the **IL-8RB** on these cells. The **chemotaxis** of N-B cells can be **inhibited** by IFN-.gamma. and IL-2, and augmented by IL-4 and IL-13, whereas TNF-.alpha. and IL-10 have no influence. The **chemotaxis** of HIV-B cells can be **inhibited** by IFN-.gamma. and IL-2, and augmented by TNF.alpha., IL-4, and IL-10, whereas IL-13 has no influence. IL-8R are expressed more abundantly on freshly isolated HIV-B cells than N-B cells (51% and 15%, resp.). The IL-8R on N-B cells can be down-regulated by IFN-.gamma., IL-2, and TNF-.alpha. (selectively on IL-8RA), and up-regulated by IL-4 and IL-13, whereas IL-10 has no influence. The IL-8R on HIV-B cells can be down-regulated by IFN-.gamma. and IL-2, and up-regulated by TNF-.alpha., IL-4, and IL-10, whereas IL-13 has no influence. Importantly, N-B cell and HIV-B cell **chemotaxis** toward IL-8 and GRO-.alpha. can be **blocked** by anti-**IL-8RB** polyclonal Ab, but not by anti-IL-8RA polyclonal Ab. Thus, IL-8 and GRO-.alpha. are important **inflammatory** mediators that stimulate the directional migration and recruitment of B lymphocytes. The migratory behavior and the expression of IL-8R on HIV-B cells and some of the reactions to Th1- and Th2-like cytokines are modified during HIV infection.

ST **chemotaxis** interleukin 8 receptor B cell; HIV infection B cell **chemotaxis** cytokine

IT Cytokines
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(GRO-.alpha.; .alpha. **chemokines** effect on **chemotaxis** and interleukin-8 receptor expression in B cells in health and HIV infection)

IT Human immunodeficiency virus
Th1 cell
Th2 cell
(**chemotaxis** and interleukin-8 receptor expression in B cells and their response to Th1- and Th2-type cytokines are modified in HIV infection)

IT Interferon .gamma.
Interleukin 10

Interleukin 13
Interleukin 2
Interleukin 4
Tumor necrosis factor .alpha.
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(**chemotaxis** and interleukin-8 receptor expression in B cells and their response to Th1- and Th2-type cytokines are modified in HIV infection)

IT B cell (lymphocyte)
**Chemotaxis**
(.alpha. **chemokines** effect on **chemotaxis** and interleukin-8 receptor expression in B cells in health and HIV infection)

IT Interleukin 8
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(.alpha. **chemokines** effect on **chemotaxis** and interleukin-8 receptor expression in B cells in health and HIV infection)

IT Interleukin 8 receptors
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(.alpha. **chemokines** effect on **chemotaxis** and interleukin-8 receptor expression in B cells in health and HIV infection)

L9 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB The mechanisms by which **chemokines** bind and signal through their receptors are complex and poorly understood. In the present study, we sought to dissect these processes and to map important functional domains of the two CXC **chemokine** (interleukin-8) receptors, CXCR1 (formally IL-8RA) and **CXCR2** (formally **IL-8RB** ), using **blocking** monoclonal antibodies (mAbs) to the receptors and a series of chimeras between CXCR1 and **CXCR2**. A panel of specific mAbs against CXCR1 or **CXCR2**, generated by immunizing mice with transfectants expressing either receptor, were shown to effectively **block** IL-8- and/or growth-related oncogene .alpha. (GRO.alpha.) -mediated ligand binding, **chemotaxis**, elastase release, and VCAM-1 binding in CXCR1 and **CXCR2** transfectants and/or human **neutrophils**. Of particular interest was an anti-CXCR1 mAb, 7D9, that **inhibited chemotaxis**, elastase release, and VCAM-1 binding but had no detectable effects on ligand binding. The epitopes of these **blocking** mAbs were mapped by using a series of CRCR1/2 chimera transfectants and synthetic peptides. Most of the anti-CRCR1 antibodies, except 7D9, mapped to the amino acid sequence WDFDDL (CXCR1 residues 10-15), and all the anti-**CXCR2** antibodies mapped to the amino acid sequence FEDFW (**CXCR2** residues 6-10). The epitope of mAb 7D9 mainly involved a region within the first 45 residues of CXCR1, and it appeared to be conformation-sensitive. These results support a model in which the binding and signaling of IL-8 with its receptor occur in at least two discrete steps involving distinct domains of the receptor. This model is consistent with the notion that discrete conformational changes of the receptor secondary to ligand binding are required to trigger various biol. responses. Moreover, the ligand binding and **chemotaxis** properties of each CXCR1/2 chimeric receptor to IL-8 and GRO.alpha. were detd. It was found that each is distinct in its ability to confer ligand binding and

chemotactic response to IL-8 and GRO-.alpha., and two conclusions could be made: (1) The N-terminal segment of CXCR1 is a dominant determinant of receptor subtype selectivity, consistent with previous studies using rabbit/human CXCR1/2 chimeras; and (2) the specificity determinant for GRO binding in **CXCR2** involves sequences in the N terminus, distal to the first 15 residues, as well as other parts of the receptor.

ACCESSION NUMBER: 1996:761983 CAPLUS
DOCUMENT NUMBER: 126:30171
TITLE: Discrete steps in binding and signaling of interleukin-8 with its receptor
AUTHOR(S): Wu, Lijun; Ruffing, Nancy; Shi, Xiaojie; Newman, Walter; Soler, Dulce; Mackay, Charles R.; Qin, Shixin
CORPORATE SOURCE: LeukoSite, Inc., Cambridge, MA, 02142, USA
SOURCE: J. Biol. Chem. (**1996**), 271(49), 31202-31209
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
SO J. Biol. Chem. (**1996**), 271(49), 31202-31209
CODEN: JBCHA3; ISSN: 0021-9258
AB The mechanisms by which **chemokines** bind and signal through their receptors are complex and poorly understood. In the present study, we sought to dissect these processes and to map important functional domains of the two CXC **chemokine** (interleukin-8) receptors, CXCR1 (formally IL-8RA) and **CXCR2** (formally **IL-8RB** ), using **blocking** monoclonal antibodies (mAbs) to the receptors and a series of chimeras between CXCR1 and **CXCR2**. A panel of specific mAbs against CXCR1 or **CXCR2**, generated by immunizing mice with transfectants expressing either receptor, were shown to effectively **block** IL-8- and/or growth-related oncogene .alpha. (GRO.alpha.) -mediated ligand binding, **chemotaxis**, elastase release, and VCAM-1 binding in CXCR1 and **CXCR2** transfectants and/or human **neutrophils**. Of particular interest was an anti-CXCR1 mAb, 7D9, that **inhibited chemotaxis**, elastase release, and VCAM-1 binding but had no detectable effects on ligand binding. The epitopes of these **blocking** mAbs were mapped by using a series of CRCR1/2 chimera transfectants and synthetic peptides. Most of the anti-CRCR1 antibodies, except 7D9, mapped to the amino acid sequence WDFDDL (CXCR1 residues 10-15), and all the anti-**CXCR2** antibodies mapped to the amino acid sequence FEDFW (**CXCR2** residues 6-10). The epitope of mAb 7D9 mainly involved a region within the first 45 residues of CXCR1, and it appeared to be conformation-sensitive. These results support a model in which the binding and signaling of IL-8 with its receptor occur in at least two discrete steps involving distinct domains of the receptor. This model is consistent with the notion that discrete conformational changes of the receptor secondary to ligand binding are required to trigger various biol. responses. Moreover, the ligand binding and **chemotaxis** properties of each CXCR1/2 chimeric receptor to IL-8 and GRO.alpha. were detd. It was found that each is distinct in its ability to confer ligand binding and chemotactic response to IL-8 and GRO-.alpha., and two conclusions could be made: (1) The N-terminal segment of CXCR1 is a dominant determinant of receptor subtype selectivity, consistent with previous studies using rabbit/human CXCR1/2 chimeras; and (2) the specificity determinant for GRO binding in **CXCR2** involves sequences in the N terminus, distal to the first 15 residues, as well as other parts of the receptor.
ST interleukin 8 receptor signal transduction **chemotaxis**

IT **Chemotaxis**
Conformation
**Neutrophil**
Signal transduction (biological)
(discrete steps in binding and signaling of interleukin-8 with receptor)
IT Interleukin 8
MGSA **chemokine**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(discrete steps in binding and signaling of interleukin-8 with receptor)

L9 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2002 ACS
AB Interleukin 8 (IL-8) is considered to be a major mediator of the **inflammatory** response. Recent evidence indicates that a direct phys. assocn. occurs between IL-8 receptors and the .alpha. subunit of guanine nucleotide regulatory protein (Gi.alpha.2) upon stimulation of human **neutrophils** by IL-8. Here, the authors identified by site-directed mutagenesis key residues within the 3 intracellular loops of the IL-8RA receptor involved in the interaction with Gi.alpha.2. They first systematically mutated, in groups of 2-4, all the residues in the 3 intracellular loops of the IL-8 type A receptor to alanine and analyzed the mutant receptors transiently expressed in 293 cells. Four residues in the second intracellular loop (Y136, L137, I139, V140) and 1 residue in the third intracellular loop (M241) were shown to be crucial for mediating calcium signaling in response to IL-8. Other residues in the second and third intracellular loops were also found to affect IL-8RA-mediated signaling, but to a lesser extent. These effects were not due to lower expression or low IL-8 binding affinities to the mutated receptors. Mutagenesis of the residues in the first intracellular loop had only weak effects on the mobilization of calcium induced by IL-8. The authors then used a coimmunopptn. protocol with anti-Gi.alpha.2 antibodies to det. the involvement of the 2 regions defined above in Gi.alpha.2 coupling to IL-8 type A receptors. Whereas the anti-Gi.alpha.2 antibodies coimmunopptd. IL-8 receptors in the wild-type cells, this interaction was lost in cells expressing mutated receptors that affected intracellular calcium mobilization. The peptides corresponding to the regions of the type A receptor crit. for Gi.alpha.2 coupling and induction of intracellular calcium mobilization were next introduced into cells expressing wild-type IL-8RA or **IL-8RB** to assess their role in coupling Gi.alpha.2 to both IL-8 receptors. The results obtained in the latter expts. suggest that the same regions of the second intracellular loop (Y136, L137, I139, V140) and of the third intracellular loop (M241) are critically involved in the coupling of both IL-8RA and **IL-8RB** to Gi.alpha.2 as well as to a downstream effector (or effectors) involved in calcium mobilization.

ACCESSION NUMBER: 1996:640396 CAPLUS
DOCUMENT NUMBER: 125:299120
TITLE: Identification of G-protein binding sites of the human interleukin-8 receptors by functional mapping of the intracellular loops
AUTHOR(S): Damaj, Bassam B.; McColl, Shaun R.; Neote, Kuldeep; Songqing, Na; Ogborn, Kevin T.; Hebert, Caroline A.; Naccache, Paul H.
CORPORATE SOURCE: Faculty of Medicine, Univ. Laval, Sainte-Foy, PQ, Can.
SOURCE: FASEB J. (**1996**), 10(12), 1426-1434
CODEN: FAJOEC; ISSN: 0892-6638
DOCUMENT TYPE: Journal

LANGUAGE: English
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CODEN: FAJOEC; ISSN: 0892-6638
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IT **Neutrophil**
Signal transduction, biological
(interleukin-8 signaling in human **neutrophils** in relation to G-protein binding sites on human interleukin-8 receptors)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(Gi2 (adenylate cyclase-**inhibiting**, 2), G-protein binding sites identification on human interleukin-8 receptors by functional mapping of intracellular loops)
IT Lymphokines and Cytokines
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(interleukin 8, interleukin-8 signaling in human **neutrophils** in relation to G-protein binding sites on human interleukin-8 receptors)
IT 182883-69-8 182883-70-1 182883-71-2 182883-72-3
RL: PRP (Properties)
(interleukin-8 signaling in human **neutrophils** in relation to G-protein binding sites on human interleukin-8 receptors)

L9 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2002 ACS
AB Interleukin 8 (IL-8) and Gro-.alpha. are members of the CXC branch of a

family of cytokines recently designated the "**chemokine**" superfamily. Recent evidence indicates that, contrary to previously held beliefs, IL-8 and Gro-.alpha. may not be perceived equivalently by **neutrophils**. In this study, we have evaluated the effects of IL-8 and Gro-.alpha. on the rate of calcium influx in human **neutrophils** and in 293 cells transfected with type A or type B IL-8 receptors. Of these two **chemokines**, only Gro-.alpha. induced an influx of calcium in **neutrophils** as judged by the sensitivity of the mobilization of calcium to the extracellular calcium chelator EGTA and to the nonselective divalent cation channel **inhibitor** SK&F 96365, as well as by manganese quenching expts. IL-8 was similarly without effect on the rate of Mn2+ influx in 293 cells transfected with IL-8 receptor A (IL-8RA) or **IL-8RB**. On the other hand, Gro-.alpha. induced an SK&F 96365-sensitive increase of the rate of Mn+2 influx in **IL-8RB**-, but not in IL-8RA-transfected 293 cells. These results indicate not only that **neutrophils** respond differently to IL-8 than they do to Gro-.alpha. but, furthermore, that the consequences of the binding of IL-8 and Gro-.alpha. to **IL-8RB** are distinct.

ACCESSION NUMBER: 1996:528399 CAPLUS
DOCUMENT NUMBER: 125:193121
TITLE: Diverging signal transduction pathways activated by interleukin 8 (IL-8) and related **chemokines** in human **neutrophils**. IL-8 and Gro-.alpha. differentially stimulate calcium influx through IL-8 receptors A and B
AUTHOR(S): Damaj, Bassam B.; McColl, Shaun R.; Neote, Kuldeep; Hebert, Caroline A.; Naccache, Paul H.
CORPORATE SOURCE: Centre de Recherche du CHUL and Department Medicine, Faculty Medicine, Universite Laval, Sainte-Foy, PQ, G1V 4G2, Can.
SOURCE: J. Biol. Chem. (**1996**), 271(34), 20540-20544
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Diverging signal transduction pathways activated by interleukin 8 (IL-8) and related **chemokines** in human **neutrophils**. IL-8 and Gro-.alpha. differentially stimulate calcium influx through IL-8 receptors A and B

SO J. Biol. Chem. (**1996**), 271(34), 20540-20544
CODEN: JBCHA3; ISSN: 0021-9258

AB Interleukin 8 (IL-8) and Gro-.alpha. are members of the CXC branch of a family of cytokines recently designated the "**chemokine**" superfamily. Recent evidence indicates that, contrary to previously held beliefs, IL-8 and Gro-.alpha. may not be perceived equivalently by **neutrophils**. In this study, we have evaluated the effects of IL-8 and Gro-.alpha. on the rate of calcium influx in human **neutrophils** and in 293 cells transfected with type A or type B IL-8 receptors. Of these two **chemokines**, only Gro-.alpha. induced an influx of calcium in **neutrophils** as judged by the sensitivity of the mobilization of calcium to the extracellular calcium chelator EGTA and to the nonselective divalent cation channel **inhibitor** SK&F 96365, as well as by manganese quenching expts. IL-8 was similarly without effect on the rate of Mn2+ influx in 293 cells transfected with IL-8 receptor A (IL-8RA) or **IL-8RB**. On the other hand, Gro-.alpha. induced an SK&F 96365-sensitive increase of the rate of Mn+2 influx in **IL-8RB**-, but not in IL-8RA-transfected 293 cells. These results indicate not only that **neutrophils** respond

differently to IL-8 than they do to Gro-.alpha. but, furthermore, that the consequences of the binding of IL-8 and Gro-.alpha. to **IL-8RB** are distinct.

ST **neutrophil** interleukin 8 signal transduction calcium; Gro alpha **chemokine neutrophil** calcium

IT **Neutrophil**
Signal transduction, biological
(interleukin-8 and Gro-.alpha. differentially stimulate calcium influx through IL-8 receptors A and B in human **neutrophils**)

IT Lymphokine and cytokine receptors
Receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(interleukin 8, A and B; interleukin-8 and Gro-.alpha. differentially stimulate calcium influx through IL-8 receptors A and B in human **neutrophils**)

IT Lymphokines and Cytokines
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(interleukin 8, interleukin-8 and Gro-.alpha. differentially stimulate calcium influx through IL-8 receptors A and B in human **neutrophils**)

IT Lymphokines and Cytokines
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(melanoma growth-stimulating activity-.alpha., interleukin-8 and Gro-.alpha. differentially stimulate calcium influx through IL-8 receptors A and B in human **neutrophils**)

IT 7439-96-5, Manganese, biological studies 7440-70-2, Calcium, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(influx; interleukin-8 and Gro-.alpha. differentially stimulate calcium influx through IL-8 receptors A and B in human **neutrophils**)

L9 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB Interleukin-8 (IL-8), one of the major mediators of the **inflammatory** response, belongs to a family of **chemokines** that includes NAP-2 (**neutrophil**-activating peptide-2) and Gro-.alpha. and whose biol. activities are directed to a great extent toward **neutrophils**. Two distinct receptors have been described with overlapping, but not identical, binding affinities for IL-8, NAP-2, and Gro-.alpha.. This study was designed to examine the intracellular pathways activated upon the occupation of each of the IL-8 receptors (IL-8R). The formation of a phys. coupling between IL-8 receptors and the .alpha.-subunit of heterotrimeric G proteins was tested in **neutrophils** by examg. the presence of the former in anti-G.alpha. immune ppts. The addn. of IL-8 to a suspension of human **neutrophils** led to a time-dependent detection of IL-8 in anti-Gi2.alpha. (raised against amino acids 159-168 (LERIAQSDYI) of Gi2.alpha.) and anti-Gt.alpha. (raised against the COOH-terminal 10 amino acids (KENLKDCGLF) of Gt.alpha.), but not anti-Gq, immunoppts. Similar results were obtained in human 293 cells stably transfected with IL-8RA or **IL-8RB**. The peptide derived from the COOH-terminal sequence of Gt **inhibited** the co-immunopptn. of IL-8R and Gi obsd. in response to the anti-Gt.alpha. and anti-Gi2.alpha. antibodies. The Gi2.alpha. peptide only **inhibited** the immunopptn. induced by the anti-Gi2.alpha. antibody. Peptides derived from Gi1.alpha. or Gi3.alpha. had no effect in this assay. The introduction of the anti-Gi2.alpha. or anti-Gt.alpha. antibodies or their neutralizing peptides, but not the Gi1.alpha. or Gi3.alpha. peptides, into 293 IL-8RA or 293 **IL-8RB** cells completely **blocked** the

calcium responses obtained upon stimulation with IL-8. These results demonstrate that the occupation of either type of IL-8 receptor leads to a phys. coupling to the .alpha.-subunit of Gi2. In addn., the use of the subunit-specific peptides identified two functionally important but distinct regions of Gi.alpha., one involved in receptor/Gi.alpha. interaction (KENLKDCGLF) and the other mediating downstream signal transmission (LERIAQSDYI). Finally, the results of this study also validate the use of the transfected 293 cell line as a model for the study of the signal transduction pathway(s) initiated by IL-8.

ACCESSION NUMBER: 1996:335064 CAPLUS
DOCUMENT NUMBER: 125:8162
TITLE: Physical association of Gi2.alpha. with interleukin-8 receptors
AUTHOR(S): Damaj, Bassam B.; McColl, Shaun R.; Mahana, Wahib; Crouch, Michael F.; Naccache, Paul H.
CORPORATE SOURCE: Cent. Rech. Rhumatol. Immunol., Univ. Laval, Sainte-Foy, PQ, G1V 4G2, Can.
SOURCE: J. Biol. Chem. (**1996**), 271(22), 12783-12789
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

SO J. Biol. Chem. (**1996**), 271(22), 12783-12789
CODEN: JBCHA3; ISSN: 0021-9258

AB Interleukin-8 (IL-8), one of the major mediators of the **inflammatory** response, belongs to a family of **chemokines** that includes NAP-2 (**neutrophil**-activating peptide-2) and Gro-.alpha. and whose biol. activities are directed to a great extent toward **neutrophils**. Two distinct receptors have been described with overlapping, but not identical, binding affinities for IL-8, NAP-2, and Gro-.alpha.. This study was designed to examine the intracellular pathways activated upon the occupation of each of the IL-8 receptors (IL-8R). The formation of a phys. coupling between IL-8 receptors and the .alpha.-subunit of heterotrimeric G proteins was tested in **neutrophils** by examg. the presence of the former in anti-G.alpha. immune ppts. The addn. of IL-8 to a suspension of human **neutrophils** led to a time-dependent detection of IL-8 in anti-Gi2.alpha. (raised against amino acids 159-168 (LERIAQSDYI) of Gi2.alpha.) and anti-Gt.alpha. (raised against the COOH-terminal 10 amino acids (KENLKDCGLF) of Gt.alpha.), but not anti-Gq, immunoppts. Similar results were obtained in human 293 cells stably transfected with IL-8RA or **IL-8RB**. The peptide derived from the COOH-terminal sequence of Gt **inhibited** the co-immunopptn. of IL-8R and Gi obsd. in response to the anti-Gt.alpha. and anti-Gi2.alpha. antibodies. The Gi2.alpha. peptide only **inhibited** the immunopptn. induced by the anti-Gi2.alpha. antibody. Peptides derived from Gi1.alpha. or Gi3.alpha. had no effect in this assay. The introduction of the anti-Gi2.alpha. or anti-Gt.alpha. antibodies or their neutralizing peptides, but not the Gi1.alpha. or Gi3.alpha. peptides, into 293 IL-8RA or 293 **IL-8RB** cells completely **blocked** the calcium responses obtained upon stimulation with IL-8. These results demonstrate that the occupation of either type of IL-8 receptor leads to a phys. coupling to the .alpha.-subunit of Gi2. In addn., the use of the subunit-specific peptides identified two functionally important but distinct regions of Gi.alpha., one involved in receptor/Gi.alpha. interaction (KENLKDCGLF) and the other mediating downstream signal transmission (LERIAQSDYI). Finally, the results of this study also validate the use of the transfected 293 cell line as a model for the study of the signal transduction pathway(s) initiated by IL-8.

IT **Neutrophil**
Signal transduction, biological
(phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)
IT G proteins (guanine nucleotide-binding proteins)
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(complexes, phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)
IT Lymphokines and Cytokines
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(interleukin 8, phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)
IT Lymphokine and cytokine receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(interleukin 8 .alpha., complexes; phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)
IT Lymphokine and cytokine receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(interleukin 8 .beta., complexes; phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)
IT Receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(interleukin 8, .alpha., complexes; phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)
IT Receptors
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(interleukin 8, .beta., complexes; phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)
IT 7440-70-2, Calcium, biological studies 111863-81-1 111863-84-4
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(phys. assocn. of G proteins with interleukin-8 receptors in **neutrophils**)

L9 ANSWER 13 OF 20 CAPLUS COPYRIGHT 2002 ACS
AB **Neutrophil** migration and activation are the cornerstones of the acute **inflammatory** response. Interleukin-8 triggers several functions of **neutrophils** in host defense: **chemotaxis**, degranulation and enzyme release, and superoxide prodn. Interleukin-8 is most potent as a chemoattractant, so **chemotaxis** is likely the most important of these functions. The effects of interleukin-8 on **neutrophils** are mediated through two receptors, IL-8RA and **IL-8RB**. To investigate the role of these receptors in **neutrophil chemotaxis**, we produced **inhibitory** antibodies to IL-8RA. These antibodies **inhibit neutrophil chemotaxis** toward IL-8 in vitro. These findings show that IL-8RA mediates a chemotactic signal in **neutrophils** and suggest that an anti-receptor strategy may be a useful approach to limit **neutrophil** migration in **inflammation**.

ACCESSION NUMBER: 1996:136645 CAPLUS
DOCUMENT NUMBER: 124:173156
TITLE: Antibodies against the N-terminus of IL-8 receptor A **inhibit neutrophil chemotaxis**
AUTHOR(S): Quan, J. M.; Martin, T. R.; Rosenberg, G. B.; Foster, D. C.; Whitmore, T.; Goodman, R. B.
CORPORATE SOURCE: Seattle VA Medical Center, Seattle, WA, USA

SOURCE: Biochem. Biophys. Res. Commun. (**1996**), 219(2), 405-11
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Antibodies against the N-terminus of IL-8 receptor A **inhibit neutrophil chemotaxis**

SO Biochem. Biophys. Res. Commun. (**1996**), 219(2), 405-11
CODEN: BBRCA9; ISSN: 0006-291X

AB **Neutrophil** migration and activation are the cornerstones of the acute **inflammatory** response. Interleukin-8 triggers several functions of **neutrophils** in host defense: **chemotaxis**, degranulation and enzyme release, and superoxide prodn. Interleukin-8 is most potent as a chemoattractant, so **chemotaxis** is likely the most important of these functions. The effects of interleukin-8 on **neutrophils** are mediated through two receptors, IL-8RA and **IL-8RB**. To investigate the role of these receptors in **neutrophil chemotaxis**, we produced **inhibitory** antibodies to IL-8RA. These antibodies **inhibit neutrophil chemotaxis** toward IL-8 in vitro. These findings show that IL-8RA mediates a chemotactic signal in **neutrophils** and suggest that an anti-receptor strategy may be a useful approach to limit **neutrophil** migration in **inflammation**.

ST antibody interleukin 8 receptor **neutrophil chemotaxis**

IT **Chemotaxis**
**Neutrophil**
(antibodies to the interleukin-8 receptor A **inhibit neutrophil chemotaxis**)

IT Antibodies
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(antibodies to the interleukin-8 receptor A **inhibit neutrophil chemotaxis**)

IT Lymphokine and cytokine receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(interleukin 8 .alpha., antibodies to the interleukin-8 receptor A **inhibit neutrophil chemotaxis**)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(interleukin 8, .alpha., antibodies to the interleukin-8 receptor A **inhibit neutrophil chemotaxis**)

L9 ANSWER 14 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB Human interleukin-8 receptors A (IL-8RA) and B (**IL-8RB**) are seven-transmembrane domain (TMD) **neutrophil chemokine** receptors with similar sequences (77% amino acid identity) and similar G protein selectivity, but markedly different selectivity for CXC **chemokines**. **IL-8RB** is selective for IL-8, growth-related oncogene .alpha. (GRO.alpha.) and **neutrophil**-activating peptide-2 (NAP-2), whereas IL-8RA is selective only for IL-8. To identify selectivity determinants, the authors made eight chimeric receptors exchanging: (1) the three main regions of sequence divergence between IL-8RA and **IL-8RB** (the N-terminal segment before TMD1, the region from TMD4 to the end of the second extracellular (e2) loop, and the C-terminal tail), and (2) the N-terminal segment of CC **chemokine** receptor 1, which does not bind CXC **chemokines**. Chimeras were tested by direct 125I-IL-8,

125I-GRO.alpha., and 125I-NAP-2 binding, heterologous competition binding, and calcium flux assays using human embryonic kidney 293 cells stably transfected with receptor DNAs. The following results were obtained: (1) chimeric receptors had binding sites for IL-8, GRO.alpha. and NAP-2 distinct from those on IL-8RA and **IL-8RB**; (2) **IL**-8, GRO.alpha. and NAP-2 bound to overlapping but distinct sites that mapped differentially to multiple domains on **IL-8RB** ; (3) high affinity radioligand binding and high agonist potency were separable functions for IL-8, GRO.alpha. and NAP-2, suggesting that the determinants of high affinity binding may not be crit. for receptor activation; and (4) determinants of GRO.alpha. and NAP-2 selectivity were found in both the N-terminal segment before TMD1 and the region from TMD4 to the end of the e2 loop of **IL-8RB**, and functioned independently of each other. Stated reciprocally, the N-terminal segment of IL-8RA was not a dominant selectivity determinant. These data suggest that both narrow and broad spectrum **chemokine** antagonists can be developed to **block** functions mediated by **IL-8RB**.

ACCESSION NUMBER: 1996:41572 CAPLUS
DOCUMENT NUMBER: 124:84572
TITLE: CXC **chemokines** bind to unique sets of selectivity determinants that can function independently and are broadly distributed on multiple domains of human interleukin-8 receptor B
AUTHOR(S): Ahuja, Sunil K.; Lee, Jennifer C.; Murphy, Philip M.
CORPORATE SOURCE: Laboratory Host Defenses, National Institutes Health, Bethesda, MD, 20892, USA
SOURCE: J. Biol. Chem. (**1996**), 271(1), 225-32
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

TI CXC **chemokines** bind to unique sets of selectivity determinants that can function independently and are broadly distributed on multiple domains of human interleukin-8 receptor B

SO J. Biol. Chem. (**1996**), 271(1), 225-32
CODEN: JBCHA3; ISSN: 0021-9258

AB Human interleukin-8 receptors A (IL-8RA) and B (**IL-8RB** ) are seven-transmembrane domain (TMD) **neutrophil** **chemokine** receptors with similar sequences (77% amino acid identity) and similar G protein selectivity, but markedly different selectivity for CXC **chemokines**. **IL-8RB** is selective for IL-8, growth-related oncogene .alpha. (GRO.alpha.) and **neutrophil**-activating peptide-2 (NAP-2), whereas IL-8RA is selective only for IL-8. To identify selectivity determinants, the authors made eight chimeric receptors exchanging: (1) the three main regions of sequence divergence between IL-8RA and **IL-8RB** (the N-terminal segment before TMD1, the region from TMD4 to the end of the second extracellular (e2) loop, and the C-terminal tail), and (2) the N-terminal segment of CC **chemokine** receptor 1, which does not bind CXC **chemokines**. Chimeras were tested by direct 125I-IL-8, 125I-GRO.alpha., and 125I-NAP-2 binding, heterologous competition binding, and calcium flux assays using human embryonic kidney 293 cells stably transfected with receptor DNAs. The following results were obtained: (1) chimeric receptors had binding sites for IL-8, GRO.alpha. and NAP-2 distinct from those on IL-8RA and **IL-8RB**; (2) **IL**-8, GRO.alpha. and NAP-2 bound to overlapping but distinct sites that mapped differentially to multiple domains on **IL-8RB** ; (3) high affinity radioligand binding and high agonist potency were

separable functions for IL-8, GRO.alpha. and NAP-2, suggesting that the determinants of high affinity binding may not be crit. for receptor activation; and (4) determinants of GRO.alpha. and NAP-2 selectivity were found in both the N-terminal segment before TMD1 and the region from TMD4 to the end of the e2 loop of **IL-8RB**, and functioned independently of each other. Stated reciprocally, the N-terminal segment of IL-8RA was not a dominant selectivity determinant. These data suggest that both narrow and broad spectrum **chemokine** antagonists can be developed to **block** functions mediated by **IL-8RB**.

ST **chemokine** interleukin 8 receptor

IT Signal transduction, biological
(**chemokine** selectivity determinants are broadly distributed on multiple domains of human interleukin-8 receptor B)

IT Gene, animal
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(GRO.alpha., **chemokine** selectivity determinants are broadly distributed on multiple domains of human interleukin-8 receptor B)

IT Lymphokines and Cytokines
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(interleukin 8, **chemokine** selectivity determinants are broadly distributed on multiple domains of human interleukin-8 receptor B)

IT Lymphokine and cytokine receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(interleukin 8 .beta., **chemokine** selectivity determinants are broadly distributed on multiple domains of human interleukin-8 receptor B)

IT Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(interleukin 8, .beta., **chemokine** selectivity determinants are broadly distributed on multiple domains of human interleukin-8 receptor B)

IT Lymphokines and Cytokines
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(**neutrophil**-activating protein 2, **chemokine** selectivity determinants are broadly distributed on multiple domains of human interleukin-8 receptor B)

L9 ANSWER 15 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB Alanine scanning mutagenesis of the charged amino acids of melanoma growth stimulating activity (MGSA) was used to identify sp. residues that are involved in binding to the human erythrocyte Duffy antigen/**chemokine** receptor (DARC) and to the type B interleukin-8 receptor (**IL-8RB**) on **neutrophils**. Receptor binding and biol. studies with the alanine scan mutants of MGSA demonstrate that MGSA binds to DARC and the **IL-8RB** through distinct binding regions. One of the MGSA mutants, E6A, binds to human erythrocytes and is able to **inhibit** malaria invasion as efficiently as wild type MGSA but has a severely reduced ability to bind to or signal through the **IL-8RB**. Mutant **chemokines** like E6A could prove to be useful therapeutically for the design of receptor **blocking** drugs that **inhibit** erythrocyte invasion by Plasmodium vivax malaria.

ACCESSION NUMBER: 1995:563119 CAPLUS

DOCUMENT NUMBER: 123:187835
TITLE: A mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria
AUTHOR(S): Hesselgesser, Joseph; Chitnis, Chetan E.; Miller, Louis H.; Yansura, Daniel G.; Simmons, Laura C.; Fairbrother, Wayne J.; Kotts, Claire; Wirth, Cindy; Gillece-Castro, Beth L.; Horuk, Richard
CORPORATE SOURCE: Genentech, Inc., South San Francisco, CA, 94080, USA
SOURCE: J. Biol. Chem. (**1995**), 270(19), 11472-6
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

TI A mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria

SO J. Biol. Chem. (**1995**), 270(19), 11472-6
CODEN: JBCHA3; ISSN: 0021-9258

AB Alanine scanning mutagenesis of the charged amino acids of melanoma growth stimulating activity (MGSA) was used to identify sp. residues that are involved in binding to the human erythrocyte Duffy antigen/ **chemokine** receptor (DARC) and to the type B interleukin-8 receptor (**IL-8RB**) on **neutrophils**. Receptor binding and biol. studies with the alanine scan mutants of MGSA demonstrate that MGSA binds to DARC and the **IL-8RB** through distinct binding regions. One of the MGSA mutants, E6A, binds to human erythrocytes and is able to **inhibit** malaria invasion as efficiently as wild type MGSA but has a severely reduced ability to bind to or signal through the **IL-8RB**. Mutant **chemokines** like E6A could prove to be useful therapeutically for the design of receptor **blocking** drugs that **inhibit** erythrocyte invasion by Plasmodium vivax malaria.

ST cytokine MGSA erythrocyte invasion **blockage** malaria; sequence mutant MGSA cytokine malaria therapeutics

IT Erythrocyte
(**blockage** to invasion of; mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

IT Malaria
Melanoma
Plasmodium vivax
Signal transduction, biological
(mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

IT Protein sequences
(of distinct binding regions; mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

IT Pharmaceuticals
(receptor **blocking**, E6A therapeutic potential use for design of; mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

IT Blood-group substances
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Duffy, receptors, residues involved in binding to; mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

IT Lymphokine and cytokine receptors
Receptors
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(interleukin 8, E6A reduced ability for binding or, **neutrophil** ; mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

IT Lymphokines and Cytokines
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(melanoma growth-stimulating activity-.alpha., amino acid sequence and therapeutic potential of; mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

IT 168043-11-6
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(amino acid sequence of mutant MGSA, therapeutic potential of; mutant of melanoma growth stimulating activity does not activate **neutrophils** but **blocks** erythrocyte invasion by malaria)

L9 ANSWER 16 OF 20 USPATFULL

AB This invention relates to the use of phenyl ureas of formulas (I) and (II) in the treatment of disease states mediated by the **chemokine**, Interleukin-8 (IL-8). The variables of (I) and (II) are defined herein. ##STR1##

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

| | | | | |
|---|---|---|---|---|
| ACCESSION NUMBER: | 2000:138404 USPATFULL | | | |
| TITLE: | IL-8 receptor antagonists | | | |
| INVENTOR(S): | Widdowson, Katherine L., King of Prussia, PA, United States | | | |
| PATENT ASSIGNEE(S): | SmithKline Beecham Corporation, Philadelphia, PA, United States (U.S. corporation) | | | |
| | NUMBER | KIND | DATE | |
| PATENT INFORMATION: | US 6133319 | | 20001017 | |
| | WO 9749680 | | 19971231 | <-- |
| APPLICATION INFO.: | US 1999-202569 | | 19990819 | (9) |
| | WO 1997-US10903 | | 19970624 | |
| | | | 19990819 | PCT 371 date |
| | | | 19990819 | PCT 102(e) date |
| | NUMBER | | DATE | |
| PRIORITY INFORMATION: | US 1996-20658 | | 19960627 (60) | |
| | US 1996-21973 | | 19960627 (60) | |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | Granted | | | |
| PRIMARY EXAMINER: | Ramsuer, Robert W. | | | |
| LEGAL REPRESENTATIVE: | Simon, Soma G., Dinner, Dara L., Kinzig, Charles M. | | | |
| NUMBER OF CLAIMS: | 12 | | | |
| EXEMPLARY CLAIM: | 1 | | | |
| LINE COUNT: | 1785 | | | |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 6133319 20001017

WO 9749680 19971231 <--

AB . . . to the use of phenyl ureas of formulas (I) and (II) in the treatment of disease states mediated by the **chemokine**, Interleukin-8 (IL-8). The variables of (I) and (II) are defined herein. ##STR1##

SUMM Many different names have been applied to Interleukin-8 (IL-8), such as **neutrophil** attractant/activation protein-1 (NAP-1), monocyte derived **neutrophil** chemotactic factor (MDNCF), **neutrophil** activating factor (NAF), and T-cell lymphocyte chemotactic factor. Interleukin-8 is a chemoattractant for **neutrophils**, basophils, and a subset of T-cells. It is produced by a majority of nucleated cells including macrophages, fibroblasts, endothelial and epithelial cells exposed to TNF, IL-1.alpha., IL-1.beta. or LPS, and by **neutrophils** themselves when exposed to LPS or chemotactic factors such as FMLP. M. Baggiolini et al, J. Clin. Invest. 84, 1045. . .

SUMM Gro.alpha., GRO.beta., GRO.gamma. and NAP-2 also belong to the **chemokine** a family. Like IL-8 these **chemokines** have also been referred to by different names. For instance Gro.alpha., GRO.beta., and GRO.gamma. have been referred to as MGSA.alpha.,. . . et al, J. Cell Physiology 129, 375 (1986) and Chang et al, J. Immunol 148, 451 (1992). All of the **chemokines** of the a-family which possess the ELR motif directly preceding the CXC motif bind to the IL-8 B receptor.

SUMM . . . NAP-2 and ENA-78 stimulate a number of functions in vitro. The have all been shown to have chemoattractant properties for **neutrophils**, while IL-8 and GROa have demonstrated T-lymphocytes, and basophiles chemotactic activity. In addition IL-8 can induce histamine release from basophils. . . from both normal and atopic individuals GRO-.alpha. and IL-8 can in addition, induce lysozomal enzyme release and respiratory burst from **neutrophils** . IL-8 has also been shown to increase the surface expression of Mac-1 (CD11b/CD18) on **neutrophils** without de novo protein synthesis. This may contribute to increased adhesion of the **neutrophils** to vascular endothelial cells. Many known diseases are characterized by massive **neutrophil** infiltration. As IL-8, Gro.alpha., GRO.beta., GRO.gamma. and NAP-2 promote the accumulation and activation of **neutrophils**, these **chemokines** have been implicated in a wide range of acute and chronic **inflammatory** disorders including psoriasis and rheumatoid **arthritis**, Baggiolini et al, FEBS Lett. 307, 97 (1992); Miller et al, Crit. Rev. Immunol. 12, 17 (1992); Oppenheim et al,. . . et al., Am. Rev. Respir. Dis. 146, 427 (1992); Donnely et al., Lancet 341, 643 (1993). In addition the ELR **chemokines** (those containing the amino acids ELR motif just prior to the CXC motif) have also been implicated in angiostasis. Strieter. . .

SUMM In vitro, IL-8, Gro.alpha., GRO.beta., GRO.gamma. and NAP-2 induce **neutrophil** shape change, **chemotaxis**, granule release, and respiratory burst, by binding to and activating receptors of the seven-transmembrane, G-protein-linked family, in particular by binding. . . 40, pp. 33-98, Birkhauser Verlag, Basel 1993. Hence, the IL-8 receptor represents a promising target for the development of novel anti-**inflammatory** agents.

SUMM Two high affinity human IL-8 receptors (77% homology) have been characterized: IL-8Ra, which binds only IL-8 with high affinity, and **IL-8Rb**, which has high affinity for IL-8 as well as for Gro.alpha., GRO.beta., GRO.gamma. and NAP-2. See Holmes et al., supra;. . .

SUMM . . . to the IL-8 a or b receptor. Therefore, conditions associated with an increase in IL-8 production (which is responsible for **chemotaxis** of **neutrophil** and T-cells subsets into the **inflammatory** site) would benefit by compounds which are **inhibitors** of IL-8 receptor binding.

SUMM This invention provides for a method of treating a **chemokine** mediated disease, wherein the **chemokine** is one which binds to an IL-8 a or b receptor and which method comprises administering an effective amount of a compound of Formula (I) or (II) or a pharmaceutically acceptable salt thereof. In particular the **chemokine** is IL-8.

SUMM This invention also relates to a method of **inhibiting** the binding of IL-8 to its receptors in a mammal in need thereof which comprises administering to said mammal an. . .

SUMM . . . and (II) may also be used in association with the veterinary treatment of mammals, other than humans, in need of **inhibition** of IL-8 or other **chemokines** which bind to the IL-8 a and b receptors. **Chemokine** mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted herein in the Methods. . .

SUMM . . . the novel compounds of Formula (II), or a pharmaceutically acceptable salt thereof, as described below, which are also useful in **inhibiting** the binding of IL-8 to its receptors in a mammal in need thereof. This invention also relates to the pharmaceutical. . . of Formula (II) and a pharmaceutically acceptable diluent or carrier. Compounds of Formula (II) are also useful for treating a **chemokine** mediated disease, wherein the **chemokine** is one which binds to an IL-8 .alpha. or .beta. receptor and which method comprises administering an effective amount of. . .

DETD . . . or unregulated IL-8 cytokine production by such mammal's cell, such as but not limited to monocytes and/or macrophages, or other **chemokines** which bind to the IL-8 a or b receptor, also referred to as the type I or type II receptor.

DETD Accordingly, the present invention provides a method of treating a **chemokine** mediated disease, wherein the **chemokine** is one which binds to an IL-8 a or b receptor and which method comprises administering an effective amount of a compound of Formula (I), or (II) or a pharmaceutically acceptable salt thereof. In particular, the **chemokines** are treating IL-8, GRO.alpha., GRO.beta., GRO.gamma., NAP-2 and ENA-78.

DETD The compounds of Formula (I) are administered in an amount sufficient to **inhibit** cytokine function, n particular treating IL-8, GRO.alpha., GRO.beta., GRO.gamma., NAP-2 and ENA-78, such that they are biologically regulated down to. . .

DETD There are many disease states in which excessive or unregulated IL-8 production is implicated in exacerbating and/or causing the disease. **Chemokine** mediated diseases include psoriasis, atopic dermatitis, **arthritis**, **asthma**, chronic obstructive pulmonary disease, adult respiratory distress syndrome, **inflammatory** bowel disease. Crohn's disease, ulcerative colitis, stroke, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, cardiac and renal **reperfusion** injury, glomerulonephritis, thrombosis, graft vs. host reaction, alzheimers disease, allograft rejections, malaria, restinosis, angiogenesis or undesired hematopoietic stem cells release.

DETD These diseases are primarily characterized by massive **neutrophil** infiltration, T-cell infiltration, or neovascular growth, and are associated with increased IL-8, GRO.alpha., GRO.beta., GRO.gamma. or

NAP-2 production which is responsible for the **chemotaxis** of **neutrophils** into the **inflammatory** site or the directional growth of endothelial cells. In contrast to other **inflammatory** cytokines (IL-1, TNF, and IL-6), IL-8, GRO.alpha., GRO.beta., GRO.gamma. or NAP-2 has the unique property of promoting **neutrophil chemotaxis**, enzyme release including but not limited to elastase release as well as superoxide production and activation. The .alpha.-**chemokines** but particularly GRO.alpha., GRO.beta., GRO.gamma. or NAP-2, working through the IL-8 type I or II receptor can promote the neovascularization of tumors by promoting the directional growth of endothelial cells. Therefore, the **inhibition** of IL-8 induced **chemotaxis** or activation would lead to a direct reduction in the **neutrophil** infiltration.

DETD Recent evidence also implicates the role of **chemokines** in the treatment of HIV infections, Littleman et al., Nature 381, pp. 661 (1996) and Koup et al., Nature 381,. . .

DETD . . . of treating, in an acute setting, as well as preventing, in those individuals deemed susceptible to, CNS injuries by the **chemokine** receptor antagonist compounds of Formula (I).

DETD . . . area, usually as a consequence of an embolus, thrombi, or local atheromatous closure of the blood vessel. The role of **inflammatory** cytokines in this are has been emerging and the present invention provides a mean for the potential treatment of these. . .

DETD . . . cytokine with proinflammatory actions, including endothelial leukocyte adhesion molecule expression. Leukocytes infiltrate into ischemic brain lesions and hence compounds which **inhibit** or decrease levels of TNF would be useful for treatment of ischemic brain injury. See Liu et al., Stoke, Vol.. . .

DETD The compounds of Formula (I) are administered in an amount sufficient to **inhibit** IL-8, binding to the IL-8 alpha or beta receptors, from binding to these receptors, such as evidenced by a reduction in **neutrophil chemotaxis** and activation. The discovery that the compounds of Formula (I) are **inhibitors** of IL-8 binding is based upon the effects of the compounds of Formulas (I) in the in vitro receptor binding assays which are described herein. The compounds of Formula (I) have been shown, in some instances, to be dual **inhibitors** of both recombinant type I and type II IL-8 receptors. Preferably the compounds are **inhibitors** of only one receptor, more preferably Type II.

DETD As used herein, the term "**chemokine** mediated disease or disease state" refers to any and all disease states in which a **chemokine** which binds to an IL-8 a or b receptor plays a role, such as but not limited to IL-8, GRO.alpha.,. . .

DETD . . . secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, **inflammatory** or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines, regardless of which cells produce them.. . . such as a macrophage and/or monocyte. Many other cells however also produce monokines, such as natural killer cells, fibroblasts, basophils, **neutrophils**, endothelial cells, brain astrocytes, bone marrow stromal cells, epideral keratinocytes and B-lymphocytes. Lymphokines are generally referred to as being produced. . .

DETD As used herein, the term "**chemokine**" refers to any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, **inflammatory**

or hematopoietic response, similar to the term "cytokine" above. A **chemokine** is primarily secreted through cell transmembranes and causes **chemotaxis** and activation of specific white blood cells and leukocytes, **neutrophils**, monocytes, macrophages, T-cells, B-cells, endothelial cells and smooth muscle cells. Examples of **chemokines** include, but are not limited to, IL-8, GRO.alpha., GRO.beta., GRO.gamma., NAP-2, ENA-78, IP-10, MIP-1a, MIP-b, PF4, and MCP 1, 2,. . .

DETD Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of **inflammation** such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The. . .

DETD The IL-8, and Gro-a **chemokine inhibitiory** effects of compounds of the present invention were determined by the following in vitro assay:

DETD . . . mixture contained .sup.125 I IL-8 (0.25 nM) or .sup.125 I Gro-a and 0.5 .mu.g/mL of IL-8Ra or 1.0 .mu.g/mL of **IL-8Rb** membranes in 20 mM Bis-Trispropane and 0.4 mM Tris HCl buffers, pH 8.0, containing 1.2 mM MgSO.sub.4, 0.1 mM EDTA,. . . After 1 hour at room temperature the plate was harvested using a Tomtec 96-well harvester onto a glass fiber filtermat **blocked** with 1% polyethylenimine/0.5% BSA and washed 3 times with 25 mM NaCl, 10 mM TrisHCl, 1 mM MgSO.sub.4, 0.5 mM. . .

DETD . . . 1 to 17, demonstrated an IC.sub.50 from about 45 to about <2 .mu.g/mL in the permissive models for IL-8 receptor **inhibition**. The compounds, N-trans-(2-benzyloxycyclohexyl)-N'-((2-benzenesulfonylamino)4-cyanophenyl))urea; N-(ethylisopropylether)-N'-(2-hydroxy-4-nitro-phenyl)urea; and N-(2-carboxyethyl)-N'-(2-hydroxy-4-nitrophenyl)urea were found to be inactive in this assay, as was the compound of Example. . .

DETD **Chemotaxis** Assay:

DETD The in vitro **inhibitory** properties of these compounds are determined in the **neutrophil chemotaxis** assay as described in Current Protocols in Immunology, vol I, Suppl 1, Unit 6.12.3., whose disclosure is incorporated herein by reference in its entirety. **Neutrophils** where isolated from human blood as described in Current Protocols in Immunology Vol I, Suppl 1 Unit 7.23.1, whose disclosure. . . washed, the membrane then stained using the Diff Quick staining protocol (Baxter Products, McGaw Park, Ill., USA). Cells which have **chemotaxed** to the **chemokine** are visually counted using a microscope. Generally, four fields are counted for each sample, these numbers are averaged to give. . . cells represent the maximum chemotactic response of the cells. In the case where a negative control (unstimulated) is desired, no **chemokine** is added to the bottom chamber. The difference between the positive control and the negative control represents the chemotactic activity. . .

DETD The compounds of this invention are tested for their ability to prevent Elastase release from human **neutrophils**. **Neutrophils** are isolated from human blood as described in Current Protocols in Immunology Vol I, Suppl 1 Unit 7.23.1. PMNs 0.88.times.10.sup.6. . .

CLM What is claimed is:

1. A method of treating a **chemokine** mediated disease state, wherein the **chemokine** binds to an IL-8 a or b receptor in a mammal, which comprises administering to said mammal an effective amount. . .

6. The method according to claim 1 wherein the mammal is afflicted with

a **chemokine** mediated disease selected from psoriasis, atopic dermatitis, **asthma**, chronic obstructive pulmonary disease, adult respiratory distress syndrome, **arthritis**, **inflammatory** bowel disease, ulcerative colitis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, stroke, cardiac and renal **reperfusion** injury, glomerulo-nephritis, thrombosis, neurotrauma, graft vs. host reaction, or allograft rejections.

L9 ANSWER 17 OF 20 USPATFULL

AB This invention relates to the novel amino substituted pyrimidine compounds of Formulas (I), (II) and (III), and pharmaceutical compositions comprising a compound of these Formulas and a pharmaceutically acceptable diluent or carrier.

This invention also relates to a method of **inhibiting** CSBP kinase and cytokines mediated by this kinase, for the treatment of cytokine mediated diseases, in mammals, by administration of a compound of Formula (I), (II) or (III). ##STR1##

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

| | | | | |
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| ACCESSION NUMBER: | 2000:98433 USPATFULL | | | |
| TITLE: | Pyrimidine compounds useful in treating cytokine mediated diseases | | | |
| INVENTOR(S): | Gallagher, Timothy F., Harlesyville, PA, United States<br>Thompson, Susan M., Phoenixville, PA, United States | | | |
| PATENT ASSIGNEE(S): | SmithKline Beecham Corporation, Philadelphia, PA, United States (U.S. corporation) | | | |
| | NUMBER | KIND | DATE | |
| PATENT INFORMATION: | US 6096748 | | 20000801 | |
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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AB This invention also relates to a method of **inhibiting** CSBP kinase and cytokines mediated by this kinase, for the treatment of cytokine mediated diseases, in mammals, by administration of. . .

SUMM . . . demonstrated to mediate a variety of biological activities

thought to be important in immunoregulation and other physiological conditions such as **inflammation** [See, e.g., Dinarello et al., Rev. Infect. Disease, 6, 51 (1984)]. The myriad of known biological activities of IL-1 include the activation of T helper cells, induction of fever, stimulation of prostaglandin or collagenase production, **neutrophil chemotaxis**, induction of acute phase proteins and the suppression of plasma iron levels.

SUMM . . . disease states in which excessive or unregulated IL-1 production is implicated in exacerbating and/or causing the disease. These include rheumatoid **arthritis**, osteoarthritis, endotoxemia and/or toxic shock syndrome, other acute or chronic **inflammatory** disease states such as the **inflammatory** reaction induced by endotoxin or **inflammatory** bowel disease; tuberculosis, **atherosclerosis**, muscle degeneration, cachexia, psoriatic **arthritis**, Reiter's syndrome, rheumatoid **arthritis**, gout, traumatic **arthritis**, rubella **arthritis**, and acute synovitis. Recent evidence also links IL-1 activity to diabetes and pancreatic .beta. cells.

SUMM Excessive or unregulated TNF production has been implicated in mediating or exacerbating a number of diseases including rheumatoid **arthritis**, rheumatoid spondylitis, osteoarthritis, gouty **arthritis** and other **arthritic** conditions; sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary **inflammatory** disease, silicosis, pulmonary sarcoisosis, bone resorption diseases, **reperfusion** injury, graft vs. host reaction, allograft rejections, fever and myalgias due to infection, such as influenza, cachexia secondary to infection. . .

SUMM . . . and/or virus replication by playing a role in maintaining T lymphocyte activation. Therefore, interference with monokine activity such as by **inhibition** of monokine production, notably TNF, in an HIV-infected individual aids in limiting the maintenance of T cell activation, thereby reducing. . . shown to activate HIV replication in monocytes and/or macrophages [See Poli, et al., Proc. Natl. Acad. Sci., 87:782-784 (1990)], therefore, **inhibition** of monokine production or activity aids in limiting HIV progression as stated above for T-cells.

SUMM . . . by IL-1, TNF, or lipopolysachharide (LPS). Human IL-8 has been shown to act on Mouse, Guinea Pig, Rat, and Rabbit **Neutrophils** . Many different names have been applied to IL-8, such as **neutrophil** attractant/activation protein-1 (NAP-1), monocyte derived **neutrophil** chemotactic factor (MDNCF), **neutrophil** activating factor (NAF), and T-cell lymphocyte chemotactic factor.

SUMM IL-8 stimulates a number of functions in vitro. It has been shown to have chemoattractant properties for **neutrophils**, T-lymphocytes, and basophils. In addition it induces histamine release from basophils from both normal and atopic individuals as well as lysozomal enzyme release and respiratory burst from **neutrophils** . IL-8 has also been shown to increase the surface expression of Mac-1 (CD11 b/CD18) on **neutrophils** without de novo protein synthesis, this may contribute to increased adhesion of the **neutrophils** to vascular endothelial cells. Many diseases are characterized by massive **neutrophil** infiltration. Conditions associated with an increased in IL-8 production (which is responsible for **chemotaxis** of **neutrophils** into the **inflammatory** site) would benefit by compounds which are suppressive of IL-8 production.

SUMM . . . wide variety of cells and tissues and these cytokines as well as other leukocyte derived cytokines are important and critical **inflammatory** mediators of a wide variety of disease states and conditions. The **inhibition** of these cytokines is of benefit in controlling, reducing and alleviating many of these disease states.

SUMM There remains a need for treatment, in this field, for compounds which are cytokine suppressive anti-**inflammatory** drugs, i.e. compounds which are capable of **inhibiting** cytokines, such as IL-1, IL-6, IL-8 and TNF.

SUMM This invention also relates to a method of **inhibiting** cytokines and the treatment of a cytokine mediated disease, in a mammal in need thereof, which comprises administering to said. . .

SUMM This invention more specifically relates to a method of **inhibiting** the production of IL-1 in a mammal in need thereof which comprises administering to said mammal an effective amount of. . .

SUMM This invention more specifically relates to a method of **inhibiting** the production of IL-8 in a mammal in need thereof which comprises administering to said mammal an effective amount of. . .

SUMM This invention more specifically relates to a method of **inhibiting** the production of TNF in a mammal in need thereof which comprises administering to said mammal an effective amount of. . .

DETD Compounds of formula (I) are capable of **inhibiting** proinflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF and are therefore of use in therapy. IL-1, IL-8 and TNF. . . a wide variety of cells and tissues and these cytokines, as well as other leukocyte-derived cytokines, are important and critical **inflammatory** mediators of a wide variety of disease states and conditions. The **inhibition** of these pro-**inflammatory** cytokines is of benefit in controlling, reducing and alleviating many of these disease states.

DETD Accordingly, in another aspect, this invention relates to a method of **inhibiting** the production of IL-1 in a mammal in need thereof which comprises administering to said mammal an effective amount of. . .

DETD . . . disease states in which excessive or unregulated IL-1 production is implicated in exacerbating and/or causing the disease. These include rheumatoid **arthritis**, osteoarthritis, endotoxemia and/or toxic shock syndrome, other acute or chronic **inflammatory** disease states such as the **inflammatory** reaction induced by endotoxin or **inflammatory** bowel disease, tuberculosis, **atherosclerosis**, muscle degeneration, multiple sclerosis, cachexia, bone resorption, psoriatic **arthritis**, Reiter's syndrome, rheumatoid **arthritis**, gout, traumatic **arthritis**, rubella **arthritis** and acute synovitis. Recent evidence also links IEL-1 activity to diabetes, pancreatic .beta. cells and Alzheimer's disease.

DETD In a further aspect, this invention relates to a method of **inhibiting** the production of TNF in a mammal in need thereof which comprises administering to said mammal an effective amount of. . .

DETD Excessive or unregulated TNF production has been implicated in mediating or exacerbating a number of diseases including rheumatoid **arthritis**, rheumatoid spondylitis, osteoarthritis, gouty **arthritis** and other **arthritic** conditions, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock

syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary **inflammatory** disease, silicosis, pulmonary sarcoisosis, bone resorption diseases, such as osteoporosis, **reperfusion** injury, graft vs. host reaction, allograft rejections, fever and myalgias due to infection, such as influenza, cachexia secondary to infection. . .

DETD . . . contemplated for treatment herein are those that produce TNF as a result of infection, or those which are sensitive to **inhibition**, such as by decreased replication, directly or indirectly, by the TNF **inhibiting**-compounds of formula (1). Such viruses include, but are not limited to HIV-1, HIV-2 and HIV-3, Cytomegalovirus (CMV), Influenza, adenovirus and. . . mammal, preferably a human, afflicted with a human immunodeficiency virus (HIV) which comprises administering to such mammal an effective TNF **inhibiting** amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

DETD . . . (I) may also be used in association with the veterinary treatment of mammals, other than in humans, in need of **inhibition** of TNF production. TNF mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted. . . Examples of such viruses include, but are not limited to, the lentivirus infections such as equine infectious anaemia virus, caprine **arthritis** virus, visna virus, or the maedi virus, or the retroviruses, such as feline immunodeficiency virus (FIV), bovine immunodeficiency virus, or. . .

DETD . . . exacerbated by excessive cytokine production, such as by IL-1 or TNF respectively, such as inflamed joints, eczema, psoriasis and other **inflammatory** skin conditions such as sunburn; **inflammatory** eye conditions including conjunctivitis; pyresis, pain and other conditions associated with **inflammation**.

DETD Another aspect of the present invention relates to a method of **inhibiting** the production of IL-8 (Interleukin-8, NAP) in a mammal in need thereof which comprises administering to said mammal an effective. . .

DETD . . . which excessive or unregulated IL-8 production is implicated in exacerbating and/or causing the disease. These diseases are characterized by massive **neutrophil** infiltration such as, psoriasis, **inflammatory** bowel disease, **asthma**, cardiac and renal **reperfusion** injury, adult respiratory distress syndrome, thrombosis and glomerulonephritis. All of these diseases are associated with increased IL-8 production which is responsible for the **chemotaxis** of **neutrophils** into the **inflammatory** site. In contrast to other **inflammatory** cytokines (IL-1, TNF, and IL-6), IL-8 has the unique property of promoting **neutrophil chemotaxis** and activation. Therefore, the **inhibition** of IL-8 production would lead to a direct reduction in the **neutrophil** infiltration.

DETD The compounds of formula (I) are administered in an amount sufficient to **inhibit** cytokine, in particular IL-1, IL-8 or TNF, production such that it is regulated down to normal levels, or in some. . .

DETD The discovery that the compounds of formula (I) are **inhibitors** of cytokines, specifically IL-1, IL-8 and TNF is based upon the effects of the compounds of formulas (I) on the. . .

DETD As used herein, the term "**inhibiting** the production of IL-1 (IL-8 or TNF)" refers to:

DETD . . . excessive in vivo levels of the cytokine (IL-1, IL-8 or TNF) in a human to normal or sub-normal levels by **inhibition** of the in

vivo release of the cytokine by all cells, including but not limited to monocytes or macrophages;

DETD c) a down regulation, by **inhibition** of the direct synthesis of the cytokine (IL-1, IL-8 or TNF) as a postranslational event; or

DETD . . . secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, **inflammatory** or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines, regardless of which cells produce them.. . . such as a macrophage and/or monocyte. Many other cells however also produce monokines, such as natural killer cells, fibroblasts, basophils, **neutrophils**, endothelial cells, brain astrocytes, bone marrow stromal cells, epideral keratinocytes and B-lymphocytes. Lymphokines are generally referred to as being produced. . .

DETD As used herein, the cytokine referred to in the phrase " **inhibition** of a cytokine, for use in the treatment of a HIV-infected human" is a cytokine which is implicated in (a). . .

DETD . . . cachectin) and since each induces similar biologic responses and binds to the same cellular receptor, both TNF-.alpha. and TNF-.beta. are **inhibited** by the compounds of the present invention and thus are herein referred to collectively as "TNF" unless specifically delineated otherwise.

DETD Compounds of Formula (I) are capable of **inhibiting** inducible proinflammatory proteins, such as COX-2, also referred to by many other names such as prostaglandin endoperoxide synthase-2 (PGHS-2) and. . . products derived from arachidonic acid, such as prostaglandins affect a wide variety of cells and tissues are important and critical **inflammatory** mediators of a wide variety of disease states and conditions. Expression of COX-1 is not effected by compounds of Formula (I). This selective **inhibition** of COX-2 may alleviate or spare ulcerogenic liability associated with **inhibition** of COX-1 thereby **inhibiting** prostoglandins essential for cytoprotective effects. Thus **inhibition** of these pro-**inflammatory** mediators is of benefit in controlling, reducing and alleviating many of these disease states. Most notably these **inflammatory** mediators, in particular prostaglandins, have been implicated in pain, such as in the sensitization of pain receptors, or edema. This aspect of pain management therefore includes treatment of neuromuscular pain, headache, cancer pain, and **arthritis** pain. Compounds of Formula (I) or a pharmaceutically acceptable salt thereof, are of use in the prophylaxis or therapy in a human, or other mammal, by **inhibition** of the synthesis of the COX-2 enzyme.

DETD Accordingly, the present invention provides a method of **inhibiting** the synthesis of COX-2 which comprises administering an effective amount of a compound of Formula (I), (II), or (III) or. . . salt thereof. The present invention also provides for a method of prophylaxis treatment in a human, or other mammal, by **inhibition** of the synthesis of the COX-2 enzyme.

DETD . . . as physicochemical stress and treatment with lipopolysaccharide or proinflammatory cytokines such as interleukin-1 and tumor necrosis factor. The cytokine biosynthesis **inhibitors** of the present invention may be determined to be potent and selective **inhibitors** of CSBP/p38/RK kinase activity by the assay as described herein. These **inhibitors** are of aid in determining the signaling pathways involvement in **inflammatory** responses. In particular, for the first time a definitive signal transduction pathway can be prescribed to the action of lipopolysaccharide in cytokine production in macrophages. In addition to those diseases

already noted, treatment of stroke, neurotrauma, cardiac and renal **reperfusion** injury, thrombosis, glomerulonephritis, diabetes and pancreatic .beta. cells, multiple sclerosis, muscle degeneration, eczema, psoriasis, sunburn, and conjunctivitis are also included.

DETD The cytokine **inhibitors** were subsequently tested in a number of animal models for anti-**inflammatory** activity. Model systems were chosen that were relatively insensitive to cyclooxygenase **inhibitors** in order to reveal the unique activities of cytokine suppressive agents. The **inhibitors** exhibited significant activity in many such in vivo studies. Most notable are its effectiveness in the collagen-induced **arthritis** model and **inhibition** of TNF production in the endotoxic shock model. In the latter study, the reduction in plasma level of TNF correlated with survival and protection from endotoxic shock related mortality. Also of great importance are the compounds effectiveness in **inhibiting** bone resorption in a rat fetal long bone organ culture system. Griswold et al., (1988) **Arthritis** Rheum. 31:1406-1412; Badger, et al., (1989) Circ. Shock 27, 51-61; Votta et al., (1994)in vitro. Bone 15, 533-538; Lee et. . .

DETD Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of **inflammation** such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The. . .

DETD The cytokine-**inhibiting** effects of compounds of the present invention are determined by the following in vitro assays:

DETD (2) Boehm, et al., 1-substituted 4-aryl-5-pyridinylimnidazoles--a new class of cytokine suppressive drugs with low 5-lipoxygenase and cyclooxygenase **inhibitory** potency. Journal Of Medicinal Chemistry 39, 3929-3937 (1996) whose disclosures are incorporated by reference herein in their entirety.

DETD The IL-8 cytokine-**inhibiting** effects of compounds of the present invention may be determined by the following in vitro assay.

DETD . . . micro plate format. Each reaction mixture containes .sup.125 I IL-8 (0.25 nM), 0.5 .mu.g/mL of IL-8Ra or 1.0 .mu.g/mL of **IL-8Rb** membranes in 20 mM Bis-Trispropane and 0.4 mM Tris HCl buffers, pH 8.0, containing 1.2 mM MgSO.sub.4, 0.1 mM EDTA,. . . After 1 hour at room temperature the plate is harvested using a Tomtec 96-well harvester onto a glass fiber filtermat **blocked** with 1% polyethylenimine/0.5% BSA and washed 3 times with 25 mM NaCl, 10 mM TrisHCl, 1 mM MgSO.sub.4, 0.5 mM. . .

DETD . . . binding assay has been extensively validated to highly correlate with the results of the bioassay. A specific and reproducible cytokine **inhibitor** binding assay was developed using soluble cystosolic fraction from THP. 1 cells and a radiolabeled compound. U.S. patent application Ser.. . .

DETD . . . with the following sequence: KRELVEPLTPSGEAPNQALLR (residues 661-681). (See Gallagher et al., "Regulation of Stress Induced Cytokine Production by Pyridinyl imidazoles: **Inhibition** of CSPB Kinase", BioOrganic & Medicinal Chemistry, to be published 1996).

DETD The following assay describes a method for determining the **inhibitory** effects of compounds of Formula (I) on human PGHS-2 protein expression in LPS stimulated human monocytes.

DETD Results: The following compounds were tested and found to be active in this assay (i.e., **inhibited** LPS induced PGHS-2 protein expression in rank order potency similar to that for **inhibiting** cytokine production as noted in assays indicated): 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole; 6-(4-Fluorophenyl)-2,3-

dihydro-5-(4-pyridinyl)imidazo[2,1-b]thiazole; and Dexamethasone.

DETD . . . found to be inactive (up to 10 uM): 2-(4-Methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-(5H)-pyrrolo[1,2-a]imidazolerolipram; phenidone and NDGA. None of the compounds tested was found to **inhibit** PGHS-1 or cPLA.sub.2 protein levels in similar experiments.

CLM What is claimed is:

7. The method according to claim 1 wherein the CSBP/RK/p38 kinase mediated disease is psoriatic **arthritis**, Reiter's syndrome, rheumatoid **arthritis**, gout, traumatic **arthritis**, rubella **arthritis** and acute synovitis, rheumatoid spondylitis, osteoarthritis, gouty **arthritis** or other **arthritic** condition.

10. The method according to claim 1 wherein the CSBP/RK/p38 kinase mediated disease is **asthma**, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary **inflammatory** disease, silicosis, or pulmonary sarcososis.

11. The method according to claim 1 wherein the CSBP/RK/p38 kinase mediated disease is **inflammatory** bowel disease, Crohn's disease, or ulcerative colitis.

14. The method according to claim 1 wherein the CSBP/RK/p38 kinase mediated disease is restenosis, cardiac and renal **reperfusion** injury, thrombosis, glomerularnephritis, or diabetes.

L9 ANSWER 18 OF 20 USPATFULL

AB This invention relates to novel carboxylic acid indole compounds and compositions for use in the treatment of disease states mediated by the **chemokine**, Interleukin-8 (IL-8).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

| | | | | |
|---|---|---|---|---|
| ACCESSION NUMBER: | 1999:113778 USPATFULL | | | |
| TITLE: | Carboxylic acid indole **inhibitors** of **chemokines** | | | |
| INVENTOR(S): | Thompson, Scott K., Phoenixville, PA, United States<br>Halbert, Stacie M., Harleysville, PA, United States<br>Widdowson, Katherine L., King of Prussia, PA, United States | | | |
| PATENT ASSIGNEE(S): | SmithKline Beecham Corporation, Philadelphia, PA, United States (U.S. corporation) | | | |
| | NUMBER | KIND | DATE | |
| PATENT INFORMATION: | US 5955492 | | 19990921 | |
| | WO 9735572 | | 19971002 | |
| APPLICATION INFO.: | US 1998-155220 | | 19980924 | (9) |
| | WO 1997-US4938 | | 19970327 | |
| | | | 19980924 | PCT 371 date |
| | | | 19980924 | PCT 102(e) date |
| | NUMBER | | DATE | |
| PRIORITY INFORMATION: | US 1996-14257 | | 19960328 (60) | |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | Granted | | | |

09/786,839

PRIMARY EXAMINER: Richter, Johann
ASSISTANT EXAMINER: Oswecki, Jane C.
LEGAL REPRESENTATIVE: Dinner, Dara L., Venetianer, Stephen, Kinzig, Charles M.
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
LINE COUNT: 1093
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Carboxylic acid indole **inhibitors** of **chemokines**
PI US 5955492 19990921
WO 9735572 19971002
AB . . . relates to novel carboxylic acid indole compounds and compositions for use in the treatment of disease states mediated by the **chemokine**, Interleukin-8 (IL-8).
SUMM Many different names have been applied to Interleukin-8 (IL-8), such as **neutrophil** attractant/activation protein-1 (NAP-1), monocyte derived **neutrophil** chemotactic factor (MDNCF), **neutrophil** activating factor (NAF), and T-cell lymphocyte chemotactic factor. Interleukin-8 is a chemoattractant for **neutrophils**, basophils, and a subset of T-cells. It is produced by a majority of nucleated cells including macrophages, fibroblasts, endothelial and epithelial cells exposed to TNF, IL-1.alpha., IL-1.beta. or LPS, and by **neutrophils** themselves when exposed to LPS or chemotactic factors such as FMLP. M. Baggiolini et al, J. Clin. Invest. 84, 1045. . .
SUMM Gro.alpha., GRO.beta., GRO.gamma. and NAP-2 also belong to the **chemokine** .alpha. family. Like IL-8 these **chemokines** have also been referred to by different names. For instance GRO.alpha.,.beta., .gamma. have been referred to as MGSA.alpha., .beta., and. . . et al, J. Cell Physiology 129, 375 (1986) and Chang et al, J. Immunol 148, 451 (1992). All of the **chemokines** of the .alpha.-family which possess the ELR motif directly preceding the CXC motif bind to the IL-8 B receptor.
SUMM . . . NAP-2 and ENA-78 stimulate a number of functions in vitro. They have all been shown to have chemoattractant properties for **neutrophils**, while IL-8 and GRO.alpha. have demonstrated T-lymphocytes, and basophiles chemotactic activity. In addition IL-8 can induce histamine release from basophils. . . from both normal and atopic individuals GRO-.alpha. and IL-8 can in addition, induce lysozomal enzyme release and respiratory burst from **neutrophils** . IL-8 has also been shown to increase the surface expression of Mac-1 (CD11b/CD18) on **neutrophils** without de novo protein synthesis. This may contribute to increased adhesion of the **neutrophils** to vascular endothelial cells. Many known diseases are characterized by massive **neutrophil** infiltration. As IL-8, Gro.alpha., GRO.beta., GRO.gamma. and NAP-2 promote the accumulation and activation of **neutrophils**, these **chemokines** have been implicated in a wide range of acute and chronic **inflammatory** disorders including psoriasis and rheumatoid **arthritis**, Baggiolini et al, FEBS Lett. 307, 97 (1992); Miller et al, Crit. Rev. Immunol. 12, 17 (1992); Oppenheim et al,. . . et al., Am. Rev. Respir. Dis. 146, 427 (1992); Donnely et al., Lancet 341, 643 (1993). In addition the ELR **chemokines** (those containing the amino acids ELR motif just prior to the CXC motif) have also been implicated in angiostasis. Strieter. . .
SUMM In vitro, IL-8, Gro.alpha., GRO.beta., GRO.gamma. and NAP-2 induce **neutrophil** shape change, **chemotaxis**, granule release, and respiratory burst, by binding to and activating receptors of the

seven-transmembrane, G-protein-linked family, in particular by binding. . . 40, pp. 33-98, Birkhauser Verlag, Basel 1993. Hence, the IL-8 receptor represents a promising target for the development of novel anti-**inflammatory** agents.

SUMM . . . to the IL-8 .alpha. or .beta. receptor. Therefore, conditions associated with an increase in IL-8 production (which is responsible for **chemotaxis** of **neutrophil** and T-cells subsets into the **inflammatory** site) would benefit by compounds which are **inhibitors** of IL-8 receptor binding.

SUMM This invention provides for a method of treating a **chemokine** mediated disease, wherein the **chemokine** is one which binds to an IL-8 .alpha. or .beta. receptor and which method comprises administering an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In particular the **chemokine** is IL-8.

SUMM This invention also relates to a method of **inhibiting** the binding of IL-8 to its receptors in a mammal in need thereof which comprises administering to said mammal an. . .

SUMM . . . Formula (I) may also be used in association with the veterinary treatment of mammals, other than humans, in need of **inhibition** of IL-8 or other **chemokines** which bind to the IL-8 a and b receptors. **Chemokine** mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted herein in the Methods. . .

DETD . . . or unregulated IL-8 cytokine production by such mammal's cell, such as but not limited to monocytes and/or macrophages, or other **chemokines** which bind to the IL-8 a or b receptor, also referred to as the type I or type II receptor.

DETD Accordingly, the present invention provides a method of treating a **chemokine** mediated disease, wherein the **chemokine** is one which binds to an IL-8 a or b receptor and which method comprises administering an effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof. In particular, the **chemokines** are IL-8, GRO.alpha., GRO.beta., GRO.gamma., NAP-2 or ENA-78.

DETD The compounds of Formula (I) are administered in an amount sufficient to **inhibit** cytokine function, in particular IL-8, GRO.alpha., GRO.beta., GRO.gamma., NAP-2 or ENA-78, such that they are biologically regulated down to normal. . .

DETD There are many disease states in which excessive or unregulated IL-8 production is implicated in exacerbating and/or causing the disease. **Chemokine** mediated diseases include psoriasis, atopic dermatitis, **arthritis**, **asthma**, chronic obstructive pulmonary disease, adult respiratory distress syndrome, **inflammatory** bowel disease, Crohn's disease, ulcerative colitis, stroke, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, cardiac and renal **reperfusion** injury, glomerulonephritis, thrombosis, graft vs. host reaction, alzheimers disease, allograft rejections, malaria, restinosis, angiogenesis or undesired hematopoietic stem cells release.

DETD These diseases are primarily characterized by massive **neutrophil** infiltration, T-cell infiltration, or neovascular growth, and are associated with IL-8, GRO.alpha., GRO.beta., GRO.gamma., or NAP-2 production which is responsible for the **chemotaxis** of **neutrophils** into the **inflammatory** site or the directional growth of endothelial cells. In contrast to other **inflammatory** cytokines (IL-1, TNF, and IL-6), with IL-8, GRO.alpha., GRO.beta., GRO.gamma., or NAP-2 has the unique property of

promoting **neutrophil chemotaxis**, enzyme release including but not limited to elastase release as well as superoxide production and activation. The a-**chemokines** but particularly, with IL-8, GRO.alpha., GRO.beta., GRO.gamma., or NAP-2, working through the IL-8 type I or II receptor can promote the neovascularization of tumors by promoting the directional growth of endothelial cells. Therefore, the **inhibition** of IL-8 induced **chemotaxis** or activation would lead to a direct reduction in the **neutrophil** infiltration.

DETD The compounds of Formula (I) are administered in an amount sufficient to **inhibit** IL-8, binding to the IL-8 alpha or beta receptors, from binding to these receptors, such as evidenced by a reduction in **neutrophil chemotaxis** and activation. The discovery that the compounds of Formula (I) are **inhibitors** of IL-8 binding is based upon the effects of the compounds of Formulas (I) in the in vitro receptor binding assays which are described herein. The compounds of Formula (I) have been shown to be dual **inhibitors** of both recombinant type I and type II IL-8 receptors. Preferably the compounds are **inhibitors** of only one receptor, preferably Type II.

DETD As used herein, the term "**chemokine** mediated disease or disease state" refers to any and all disease states in which a **chemokine** which binds to an IL-8 .alpha. or b receptor plays a role, such as but not limited to with IL-8,. . .

DETD . . . secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, **inflammatory** or hematopoietic response. A cytokine includes, but is not limited to, monokines and lymphokines, regardless of which cells produce them.. . . such as a macrophage and/or monocyte. Many other cells however also produce monokines, such as natural killer cells, fibroblasts, basophils, **neutrophils**, endothelial cells, brain astrocytes, bone marrow stromal cells, epideral keratinocytes and B-lymphocytes. Lymphokines are generally referred to as being produced. . .

DETD As used herein, the term "**chemokine**" refers to any secreted polypeptide that affects the functions of cells and is a molecule which modulates interactions between cells in the immune, **inflammatory** or hematopoietic response, similar to the term "cytokine" above. A **chemokine** is primarily secreted through cell transmembranes and causes **chemotaxis** and activation of specific white blood cells and leukocytes, **neutrophils**, monocytes, macrophages, T-cells, B-cells, endothelial cells and smooth muscle cells. Examples of **chemokines** include, but are not limited to with IL-8, GRO.alpha., GRO.beta., GRO.gamma., NAP-2, ENA-78, IP-10, MIP-1.alpha., MIP-.beta., PF4, and MCP 1,. . .

DETD Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of **inflammation** such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The. . .

DETD The IL-8 cytokine-**inhibiting** effects of compounds of the present invention were determined by the following in vitro assay:

DETD . . . mixture contained .sup.125 I IL-8 (0.25 nM) or .sup.125 I Gro-a and 0.5 .mu.g/mL of IL-8Ra or 1.0 .mu.g/mL of **IL-8Rb** membranes in 20 mM Bis-Trispropane and 0.4 mM Tris HCl buffers, pH 8.0, containing 1.2 mM MgSO.sub.4, 0.1 mM EDTA,. . . After 1 hour at room temperature the plate was harvested using a Tomtec 96-well harvester onto a glass fiber filtermat **blocked** with 1%

polyethylenimine/0.5% BSA and washed 3 times with 25 mM NaCl, 10 mM TrisHCl, 1 mM MgSO.sub.4, 0.5 mM. . .

DETD Compounds of Formula (I) as exemplified by Examples 1 to 16 all showed a positive **inhibition** in this assay from a range of 4 .mu.M to about 50 .mu.Molar.

DETD **Chemotaxis** Assay:

DETD The in vitro **inhibitory** properties of these compounds were determined in the **neutrophil chemotaxis** assay as described in Current Protocols in Immunology, Vol I, Suppl 1, Unit 6.12.3., whose disclosure is incorporated herein by reference in its entirety. **Neutrophils** where isolated from human blood as described in Current Protocols in Immunology Vol I, Suppl 1 Unit 7.23.1, whose disclosure. . . the membrane was then stained using the Diff Quick staining protocol (Baxter Products, McGaw Park, Ill., USA). Cell which had **chemotaxed** to the **chemokine** were visually counted using a microscope. Generally, four fields where counted for each sample, these number where averaged to give. . . cells represent the maximum chemotactic response of the cells. In the case where a negative control (unstimulated) was desired, no **chemokine** was added to the bottom chamber. The difference between the positive control and the negative control represents the chemotactic activity. . .

DETD The compounds of this invention where tested for their ability to prevent Elastase release from human **neutrophils**. **Neutrophils** where isolated from human blood as described in Current Protocols in Immunology Vol I, Suppl 1 Unit 7.23.1. PMNs 0.88.times.10.sup.6. . .

CLM What is claimed is:

9. A method of treating a **chemokine** mediated disease state, wherein the **chemokine** binds to an IL-8 .alpha. or .beta. receptor in a mammal, which comprises administering to said mammal an effective amount. . .

10. The method according to claim 9 wherein the **chemokine** is IL-8.

. . . method according to claim 9 wherein the mammal is afflicted with an Il-8 mediated disease selected from psoriasis, atopic dermatitis, **arthritis**, **asthma**, chronic obstructive pulmonary disease, adult respiratory distress syndrome, **inflammatory** bowel disease, Crohn's disease, ulcerative colitis, stroke, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, cardia and renal **reperfusion** injury, glomerulonephritis, thrombosis, graft vs. host reaction, allograft rejections, and malaria.

12. A method of treating **inflammation** in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound of Formula. . .

13. A method of treating **asthma** in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound of Formula. . .

L9 ANSWER 19 OF 20 USPATFULL

AB gpD protein, the major subunit of the Duffy blood group antigenic system, has been isolated. gpD protein contains the receptor, by which P. vivax enters red cells and causes malaria. gpD has significant sequence homology with human and rabbit interleukin-8 receptors and, therefore, gpD protein likely is a new class of chemoattractant cytokines receptor. gpD protein cDNA has a quasi-total homology with a human hippocampus cDNA clone HHCMF86 and, therefore, gpD protein or a

homologous protein may be present as a neuropeptide receptor in brain. gpD protein is present in all red cell progenitors and it may be a receptor for cell proliferation and/or differentiation. gpD protein cDNA identifies in human kidney a mRNA of the same size as the bone marrow. Since the kidney is not and has no potential to become an erythropoietic organ, this putative chemoattractant receptor may have essential renal functions. gpD protein has therapeutic value in the prevention of malaria and in the regulation of erythrocyte, neural and renal functions and can be combined with physiologically acceptable diluents to yield a therapeutic agent suitable for these purposes. Peptides corresponding to a portion of gpD protein that contains the receptor also have been synthesized. Such peptides have therapeutic usefulness identical to that of gpD protein. gpD protein and such peptides also have utility in the production of therapeutics, e.g., antibodies, complementary peptides, etc., which are also useful to treat malaria and regulate essential erythrocyte, neural and renal functions.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

| | |
|---|---|
| ACCESSION NUMBER: | 97:101459 USPATFULL |
| TITLE: | Cloning of duffy blood group antigen, gpD |
| INVENTOR(S): | Pogo, Angel Oscar, Pelham, NY, United States<br>Chaudhuri, Asok, Rego Park, NY, United States |
| PATENT ASSIGNEE(S): | New York Blood Center, Inc., New York, NY, United States (U.S. corporation) |

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 5683696 | | 19971104 | <-- |
| APPLICATION INFO.: | US 1995-486670 | | 19950607 (8) | |
| RELATED APPLN. INFO.: | Division of Ser. No. US 1993-140797, filed on 21 Oct 1993, now patented, Pat. No. US 5578714 | | | |

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|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | Granted |
| PRIMARY EXAMINER: | Cunningham, Thomas M. |
| LEGAL REPRESENTATIVE: | Sprung Kramer Schaefer & Briscoe |
| NUMBER OF CLAIMS: | 18 |
| EXEMPLARY CLAIM: | 1 |
| NUMBER OF DRAWINGS: | 9 Drawing Figure(s); 7 Drawing Page(s) |
| LINE COUNT: | 984 |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5683696 19971104 <--

DETD . . . structure-function of this novel red cell membrane protein that might exist in other cell types and may function as a **chemokine** receptor, and (iv) the role of gpD protein as the receptor for P. vivax merozoite invasion.

DETD . . . U.S. Pat. No. 5,101,017, have disclosed a monoclonal antibody specific for gpD protein (hereinafter "the Rubinstein antibody"). The Rubinstein antibody **blocks** the penetration of the P. vivax malaria parasite into human red blood cells by virtue of effective **blocking** of the target molecule of the P. vivax malaria parasite. It is likely that the Rubinstein antibody has a combining. . . vivax infection, to induce anti-idiotypic responses, as alluded to above, that protect against these parasites, and directly in vivo to **block** the red cell receptors for the parasites. Details of these and other uses of the Rubinstein et al. antibody are. . .

DETD . . . receptors on erythrocytes. This is consistent with a recent report suggesting that the Duffy blood group antigen and the erythrocyte **chemokine** receptor are the same protein. R. Horuk et al., "A

Receptor for the Malarial Parasite Plasmodium vivax: The Erythrocyte **Chemokine** Receptor", Science, 261, 1182 (1993). The erythrocyte receptor apparently differs from the IL-8 receptors, IL-8RA and **IL-8RB** on **neutrophils**. The erythrocyte receptor binds a family of chemotactic and proinflammatory soluble peptides, including IL-8, melanoma growth stimulatory activity (MGSA), monocyte. . . secretion of these proteins. For example, it has been postulated that the erythrocyte receptor acts as a scavenger for certain **inflammatory** mediators, including IL-8. Administration of gpD protein (or the inventive synthetic peptides), therefore, would be expected to enhance scavenging of IL-8, thereby, lessening any IL-8 induced **inflammation**. For this purpose, the inventive vaccine, as described above, is suitable as a therapeutic agent.

DETD Proteins that are complementary to gpD protein or the inventive synthetic peptides, e.g., antibodies specific to gpD, will **block** the natural receptor(s) and, consequently, will also have the therapeutic utilities outlined above. In the preparation of such complementary proteins,. . .

DETD Pe 1 peptide was obtained by sequencing the non-fractionated CNBr digest using the O-phthalaldehyde (OPA) **blocking** reagent (see, A. W. Brauer et al., Anal. Biochem., 137, 134 (1983)). Pe 5 peptide was the partial sequence of. . .

DETD . . . et al., Science, 253, 1278 (1991); and P. M. Murphy et al., Science, 253, 1280 (1991). If dpD protein bind **chemokines** and has the ability to activate a signal transduction cascade, this gives rise to gpD protein as a new class of pro-**inflammatory** mediators. Thus, gpD protein is not present in white blood cells, since a rabbit polyclonal antibody (anti-gpD) against purified and. . .

L9 ANSWER 20 OF 20 USPATFULL

AB gpD protein, the major subunit of the Duffy blood group antigenic system, has been isolated. gpD protein contains the receptor, by which P. vivax enters red cells and causes malaria. gpD has significant sequence homology with human and rabbit interleukin-8 receptors and, therefore, gpD protein likely is a new class of chemoattractant cytokines receptor. gpD protein cDNA has a quasi-total homology with a human hippocampus cDNA clone HHCMF86 and, therefore, gpD protein or a homologous protein may be present as a neuropeptide receptor in brain. gpD protein is present in all red cell progenitors and it may be a receptor for cell proliferation and/or differentiation. gpD protein cDNA identifies in human kidney a mRNA of the same size as the bone marrow. Since the kidney is not and has no potential to become an erythropoietic organ, this putative chemoattractant receptor may have essential renal functions. gpD protein has therapeutic value in the prevention of malaria and in the regulation of erythrocyte, neural and renal functions and can be combined with physiologically acceptable diluents to yield a therapeutic agent suitable for these purposes. Peptides corresponding to a portion of gpD protein that contains the receptor also have been synthesized. Such peptides have therapeutic usefulness identical to that of gpD protein. gpD protein and such peptides also have utility in the production of therapeutics, e.g., antibodies, complementary peptides, etc., which are also useful to treat malaria and regulate essential erythrocyte, neural and renal functions.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 96:109080 USPATFULL

TITLE: DNA encoding Duffy 9pd protein

INVENTOR(S): Pogo, Angel O., Pelham, NY, United States

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|---|---|
| PATENT ASSIGNEE(S): | Chaudhuri, Asok, Rego Park, NY, United States<br>New York Blood Center, Inc., New York, NY, United States (U.S. corporation) |

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 5578714 | | 19961126 | <-- |
| APPLICATION INFO.: | US 1993-140797 | | 19931021 (8) | |

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|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | Granted |
| PRIMARY EXAMINER: | Cunningham, Thomas M. |
| LEGAL REPRESENTATIVE: | Sprung Horn Kramer & Woods |
| NUMBER OF CLAIMS: | 3 |
| EXEMPLARY CLAIM: | 1 |
| NUMBER OF DRAWINGS: | 9 Drawing Figure(s); 7 Drawing Page(s) |
| LINE COUNT: | 937 |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

PI US 5578714 19961126 <--

DETD . . . structure-function of this novel red cell membrane protein that might exist in other cell types and may function as a **chemokine** receptor, and (iv) the role of gpD protein as the receptor for P. vivax merozoite invasion.

DETD . . . U.S. Pat. No. 5,101,017, have disclosed a monoclonal antibody specific for gpD protein (hereinafter "the Rubinstein antibody"). The Rubinstein antibody **blocks** the penetration of the P. vivax malaria parasite into human red blood cells by virtue of effective **blocking** of the target molecule of the P. vivax malaria parasite. It is likely that the Rubinstein antibody has a combining. . . vivax infection, to induce anti-idiotypic responses, as alluded to above, that protect against these parasites, and directly in vivo to **block** the red cell receptors for the parasites. Details of these and other uses of the Rubinstein et al. antibody are. . .

DETD . . . receptors on erythrocytes. This is consistent with a recent report suggesting that the Duffy blood group antigen and the erythrocyte **chemokine** receptor are the same protein. R. Horuk et al., "A Receptor for the Malarial Parasite Plasmodium vivax: The Erythrocyte **Chemokine** Receptor", Science, 261, 1182 (1993). The erythrocyte receptor apparently differs from the IL-8 receptors, IL-8RA and **IL-8RB** on **neutrophils**. The erythrocyte receptor binds a family of chemotactic and proinflammatory soluble peptides, including IL-8, melanoma growth stimulatory activity (MGSA), monocyte. . . secretion of these proteins. For example, it has been postulated that the erythrocyte receptor acts as a scavenger for certain **inflammatory** mediators, including IL-8. Administration of gpD protein (or the inventive synthetic peptides), therefore, would be expected to enhance scavenging of IL-8, thereby, lessening any IL-8 induced **inflammation**. For this purpose, the inventive vaccine, as described above, is suitable as a therapeutic agent.

DETD Proteins that are complementary to gpD protein or the inventive synthetic peptides, e.g., antibodies specific to gpD, will **block** the natural receptor(s) and, consequently, will also have the therapeutic utilities outlined above. In the preparation of such complementary proteins,. . .

DETD Pe 1 peptide was obtained by sequencing the non-fractionated CNBr digest using the O-phthalaldehyde (OPA) **blocking** reagent (see, A. W. Brauer et al., Anal. Biochem., 137, 134 (1983)). Pe 5 peptide was the partial sequence of. . .

DETD . . . et al., Science, 253, 1278 (1991); and P. M. Murphy et al.,

Science, 253, 1280 (1991). If dpD protein bind **chemokines** and has the ability to activate a signal transduction cascade, this gives rise to gpD protein as a new class. . .